THE ROLE OF THALIDOMIDE ON CACHEXIA DURING EXPERIMENTAL SKIN CARCINOGENESIS IN MICE

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Abstract

We investigated the effect of thalidomide on cachexia and TNF-α serum levels during experimental skin carcinogenesis in mice. Female mice were divided into four groups: 1) DMBA (dissolved in acetone) induced tumorigenesis; 2) DMBA and Thalidomide (dissolved in DMSO); 3) DMBA and DMSO; and 4) Acetone. Body weight was measured once a week. Euthanasia was performed 14 weeks later, when blood was collected for the dosage of TNF-α serum levels. Mice with DMBA induced tumorigenesis had a significant loss of body weight when compared to acetone treated animals, starting at the third week and lasting the whole experiment. But there was no difference among Thalidomide treated and the others DMBA control animals, regarding body weight. High TNF-α serum levels were associated with the development of cachexia in mice during the process of experimental skin tumorigenesis. However, there was not a significant difference in the TNF-α serum levels when compared control mice and thalidomide treated mice. These results suggest that thalidomide does not interfere with skin tumorigenesis, cachexia and serum TNF-α levels in Balb/C mice. In addition, high TNF-α serum levels are associated to weight loss during experimental carcinogenesis.

Keywords

Cachexia. TNF-α. Thalidomide. Skin chemical carcinogenesis. DMBA.

1 INTRODUCTION

The word "cachexia" is derived from the Greek "katos", meaning "bad", and "hexis", meaning "condition" (TISDALE, 1997). The cachexia syndrome is characterized by weight loss, anorexia, anemia, and depletion of lean body weight and has been recognized as a poor prognostic sign during the development of several diseases. It occurs in a number of disease states, including cancer, acquired immunodeficiency syndrome (AIDS), major trauma, surgery, malabsorption, and severe sepsis (TRACEY et al., 1988; TISDALE, 1997; SHARMA; ANKER, 2002). Progressive wasting is common in many types of cancer and is one of the most important factors leading to the early death of cancer patients. Although anorexia frequently accompanies cachexia, it has been difficult to establish a simple cause-and-effect relationship, and nutritional supplementation is not able to reverse effectively the process of cachexia. Historical explanations for the causes of cancer induced wasting have been varied. Some experts have claimed that the dominant cause of weight loss is heightened energy demands, attributable to both the needs for tumour growth and tumour triggered changes in metabolism of tissues distant to the malignant process (STROUD, 2005). There is increasing evidence that the immune system (in particular inflammatory cytokines) plays an important role in the development of cachexia. Cancer patients have been shown to have elevated production of proinflammatory cytokines, either produced by the tumour itself or released as part of a host response. These cytokines influence appetite, metabolic demands, and relative substrate utilization. The cytokine considered to be the most relevant to this process is tumor necrosis factor alpha (TNF-α) or cachectin, although other mediators such as interleukin 1 (IL-1), IL-6 and interferon gamma have also been implicated (TRACEY et al., 1988; YONEDA et al., 1991; STRASSMAN et al., 1992; SHARMA; ANKER, 2002; TISDALE, 2004; STROUD, 2005).

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TNF-α is a pleiotropic cytokine, produced primarily by monocytes and macrophages, which plays an important role in host immune responses. It acts on many cell types including endothelial cells, leukocytes, and fibroblast. Dysregulation of TNF-α may contribute to the pathogenesis of various diseases, such as acute inflammation in erythema nodosum leprosum, and tuberculosis meningitis, tissue destruction in rheumatoid arthritis, cachexia in AIDS and cancer patients, and septic shock (DENG et al., 2003). It enhances neo-angiogenesis, interacts with other proliferative cytokines such as IL-6, and contributes to many of the systemic symptoms of advanced malignancy. TNF-α was originally considered to have activity against malignant disease. However, recent studies suggest that TNF-α may also act as an endogenous tumor promoter, as TNF-knockout mice are resistant to skin carcinogenesis chemically induced (MOORE et al., 1999; SUGANUMA et al., 1999; THOMAS; KANTARJIAN et al., 2000; SCOTT et al., 2003).

In a human squamous cell carcinoma of the maxilla grown as a xerograft in nude mice, anti-TNF-α antibodies reversed partially, but did not normalize completely the body weight. A similar result has been obtained in a single study using pentoxifylline, which has been reported to decrease TNF-α mRNA levels in cancer patients (GOLDBERG et al., 1995; TISDALE, 1997). It was also demonstrated that TNF-α antibodies attenuate the development of cachexia in mice tumor models (SHERRY et al., 1989).

Improved understanding of the triggers and responses underlying cancer cachexia offers new targets for potential therapeutic intervention (STROUD, 2005). Thalidomide is an important TNF-α inhibitor (BAUDITZ et al., 2002). It inhibits tumour necrosis factor production in stimulated human peripheral monocytes (SAMPAIO et al., 1991), and this inhibitory action was thought to be due to enhancement of TNF-α mRNA degradation (MOREIRA et al., 1993). Thalidomide shows strong potential as an anticancer agent, which has a grat number of actions, including inhibition of angiogenesis, modulation of adhesion molecules, inhibition of cyclooxygenase 2, and stimulation of immune responses (KUMAR et al., 2002; LIN et al., 2003; ELEUTHERAKIS-PAPAIAKOVOU et al., 2004; STROUD, 2005). Owing to these unique mechanisms of action and favorable side effect profile relative to chemotherapy, the study of thalidomide as a potential anti-tumor agent has intensified (AARESTRUP et al., 1995; CALABRESE; FLEISCHER, 2000; RICHARDSON et al., 2002; FRANKS et al., 2004; SLEIJFER et al., 2004).

Some studies showed that thalidomide is well tolerated and effective at attenuating loss of weight and lean body mass in patients with cachexia due to terminal cancer (BRUERA et al., 1999). Recently, it was demonstrated that thalidomide is safe and may be effective in attenuating severe weight loss in patients with advanced pancreatic cancer. This may also grant benefit in terms of improved physical function (STROUD, 2005; GORDON et al., 2005). However the mechanism of action of thalidomide on cachexia is not completely understood.

One of the best animal models characterized for chemical carcinogenesis studies is the mouse skin (STOLER et al., 1993; YUSPA, 1998; BALMAIN, 2000; HIRST; BALMAIN, 2004). Epidermal neoplasia can be induced in this model by two different protocols, complete carcinogenesis and two-stage carcinogenesis. In the first protocol, a large single dose or, more commonly, multiple low-dose application of a genotoxic carcinogen e.g., 7,12-dimethylbenz[a]nthracene (DMBA) is used to induce tumors on the backs of susceptible mice. According to Park and Kim (1989), DMBA is very potent and can be considered a complete carcinogen, as it possesses properties of initiating, promoting and progressor agents (PITOT; DRAGAN, 1991), and it is more effective than the two stage protocols (PARK; KIM, 1989). This treatment usually results in the development of numerous benign papillomas, some of which progress to malignant squamous cell carcinomas (OWENS et al., 1999; ZOUMPOURLIS et al., 2003).

Skin tumorigenesis DMBA-induced in mice is a classical model used to test therapeutical procedures against cancer (OWENS et al., 1999; ZOUMPOURLIS et al., 2003). In the present study we used this model to investigate the effect of thalidomide on cachexia and on TNF-α serum levels.

2 MATERIAL AND METHODS

2.1 ANIMALS

All procedures were performed according to the regulations of the institutional ethical committee. Forty female Balb/c mice, 6-8 weeks old, weighting approximately 19g, were obtained from the animal quarters of CBR (Centro de Biologia da Reprodução – Universidade Federal de Juiz de Fora, Brasil). Animals were maintained in a controlled environment under conditions of constant temperature and humidity with an alternating 12-h light/dark cycle, housed individually and were fed on rat chow pellets and water ad libitum.

The mice were randomly divided into four groups of ten animals each:

- Group 1: DMBA (control group)
- Group 2: DMBA + Thalidomide
- Group 3: DMBA + DMSO (control group)
- Group 4: Acetone (control group)

2.2 DMBA INDUCED SKIN TUMORIGENESIS

The dorsal skin of each mouse was shaved one day before the initiation and promotion with 7,12-dimethylbenz[a]nthracene (DMBA, Sigma Chemical Co.). Tumorigenesis was induced by twice-

weekly topical applications, in the back skin of mice, of 100µL of 0.5% DMBA in acetone solution, during 14 weeks consecutively. In group 4, only acetone (100 µL) was applied to the back skin of mice following the same protocol of group 1.

2.3 THALIDOMIDE ADMINISTRATION

Animals from the group 2 (DMBA + Thalidomide) received daily subcutaneous injections of 0,1 mL of thalidomide (kindly provided by Dr. Elizabeth Sampaio – Fundação Oswaldo Cruz, Rio de Janeiro, Brasil) at a dose of 30 mg/Kg body weight, since the first day of the experiment. Thalidomide was dissolved in dimethyl sulfoxide (DMSO, Fluka Chemika) and further dilutions were made in sterile acidified saline. The final concentration of DMSO was 2% (AARESTRUP et al., 1995). The group 3 (DMBA + DMSO) received saline and identical concentrations of DMSO daily.

2.4 ASSESSMENT OF BODY WEIGHT AND HISTOPATHOLOGICAL STUDY

Body weight was measured once a week during the whole experiment. Animals were treated during 14 weeks. At the last week, after anesthesia (Ketamine 100mg/Kg and Xylazine 10 mg/Kg via intraperitonial injection), it was performed cardiac puncture, the blood sample was centrifuged and the serum was frozen for dosage of TNF-α by ELISA. Mice were euthanized through excessive anesthesia or exsanguination.

The tumors were excised, fixed in 10% neutral-buffered formalin, processed routinely, and embedded in paraffin. Paraffin sections (5µm thick) were stained with hematoxylin and eosin for histological examination.

2.5 SERUM TNF-α MEASUREMENT

Blood samples for TNF-α determination were obtained as described above. Mice were anesthetized and bled by cardiac puncture. Blood was allowed to clot at room temperature and then was centrifuged and the serum was aliquoted and stored at -70ºC until use. The TNF-α serum levels were determined by ELISA according to the manufacturer's (Pharmingen) instructions. Samples were plated in duplicate.

2.6 STATISTICAL ANALYSIS

The statistical significance of the experimental results was determined by one-way analysis of variances (ANOVA) followed by LSD test. For all analyses, $p<0.05$ was accepted as a significant probability level.

3 RESULTS

All the animals presented skin tumors, usually with multiple lesions. The histopathological analysis showed that 100% of the DMBA treated animals had tumoral lesions, including group 2 (DMBA + Thalidomide).

As shown in Fig 1, mice with DMBA induced tumorigenesis had a significant loss of body weight when compared to acetone treated animals, starting at the third week and lasting the whole experiment. But there was no difference among Thalidomide treated and the others DMBA control animals, regarding body weight.

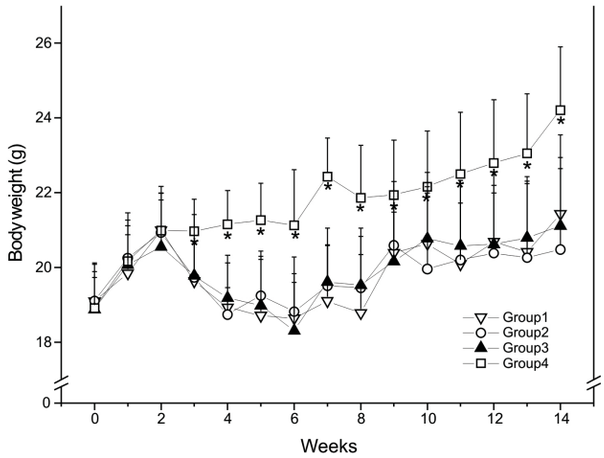

Figure 1: Body weight (g) of the animals. Group 1 (DMBA), group 2 (DMBA + Thalidomide), group 3 (DMBA + DMSO), and group 4 (Acetone). Values are expressed as mean ± standard error. *$p < 0.05$ versus all DMBA treated groups (analysis of variance with LSD test).

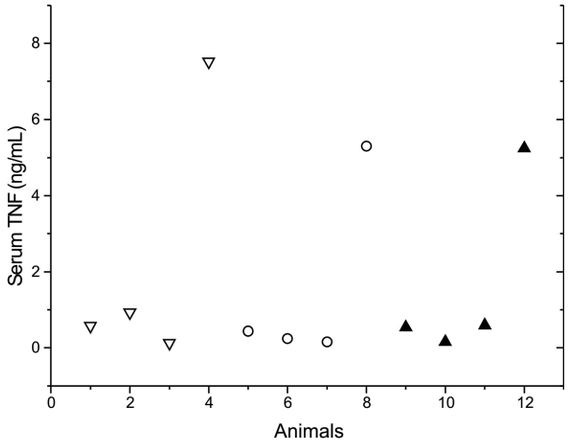

Figure 2: TNF-α serum levels. Group 1 (▽), group 2 (o), and group 3 (▲). The results are expressed as a mean of two different experiments, with four mice in each group.

Searching to examine the effects of thalidomide on TNF-α production and the effects of this cytokine on cachexia, TNF-α serum levels were determined (Figure 2). The serum from the control mice

did not contain detectable TNF-α as determined by ELISA, in which the lower limit of detection was 10 pg/ml. High TNF-α serum levels were associated with the development of cachexia in mice during the process of experimental skin tumorigenesis. However, there was not a significant difference in the TNF-α serum levels when compared control mice and thalidomide treated mice.

4 DISCUSSION

All DMBA-treated mice developed skin tumors, including group 2 (DMBA + thalidomide), what suggests that thalidomide was not effective in preventing or treating skin tumors in Balb/c mice. Acetone treated group did not present tumor lesions. All mice developed multiple skin lesions recognized as squamous papillomas which progressed to squamous cell carcinomas. Malignant conversion was characterized by invasion through the basement membrane and migration into the underling stroma and involved changes in cell-cell and cell-matrix interactions. These results are according to previous works (DUBOWSKI et al., 1998; YUSPA, 1998; WOODWORTH et al., 2004; GLICK; YUSPA, 2005).

Thalidomide is a glutamic acid derivative initially introduced as a sedative hypnotic nearly forty years ago. It was withdrawn following numerous reports linking it to a characteristic pattern of congenital abnormalities in babies born to mothers who used the drug for mourning sickness. It has gradually been re-introduced into clinical practice over the past two decades, under strict regulation, since it was found to be useful in the management of erythema nodosum leprosum and HIV wasting syndrome. Thalidomide is a putative anti-angiogenesis and immunomodulatory agent that has demonstrated activity in various dermatologic and rheumatologic conditions in addition to Crohn's disease, and is being investigated extensively in the management of advanced cancer (THOMAS; KANTARJIAN, 2000; GERSON-CWILICH et al., 2001; KUMAR et al., 2002; RICHARDSON et al., 2002; WINES et al., 2002; ELEUTHERAKIS-PAPAIAKOVOU et al., 2004). The mechanisms of its antineoplastic effects continue to be the focus of ongoing research. It possesses anti-TNF-α activity, which has led to its evaluation in several inflammatory and cachetic states (AARESTRUP et al., 1995; BRUERA et al., 1999; BAUDITZ et al., 2002; KUMAR et al., 2002; LIN et al., 2003; GORDON et al., 2005; STROUD, 2005).

All DMBA treated mice lost weight (cachexia) when compared to acetone group. But when we compared groups 1, 2 and 3, there were no significant differences. The later groups also had high TNF-α serum levels, suggesting that TNF-α is associated to weight loss in cancer cachexia, and confirming results from previous works (TRACEY et al., 1988; SHERRY et al., 1989; YONEDA et al., 1991). Opposing to the hypothesis that thalidomide inhibits TNF-α production, in the present work, we observed that thalidomide did not influence TNF-α serum levels during the process of tumorigenesis, as all DMBA treated mice had detectable levels of this cytokine in serum, with no significant difference among them.

Thalidomide is capable of reducing TNF-α both *in vivo* and *in vitro* and also has anti-angiogenic effects (SAMPAIO et al., 1991; TRAMONTANA et al., 1995). TNF-α is one of the likely mediators of cachexia. Because of its property to reduce the TNF-α synthesis, thalidomide is also used in patients with pulmonary tuberculosis and in AIDS patients whose condition is similar to cancer associated cachexia (TRAMONTANA et al., 1995; HASLETT et al., 1997). Bruera et al. (1999) demonstrated that low-dose thalidomide is capable of improving the subjective symptoms of cachexia in patients with terminal cancer, particularly improving patient's appetite, and their overall sensation of well being. In another experiment, Boasberg et al. (2000) reported that thalidomide induced cessation of weight loss and decreased nocturnal awakenings in advanced cancer patients with cachexia. It is also known that thalidomide is well tolerated and effective at attenuating loss of weight and lean body mass in patients with cachexia due to advanced pancreatic cancer (GORDON et al., 2005; STROUD, 2005). However the mechanism of action of thalidomide on cachexia was not completely elucidated.

We used the classical model of skin carcinogenesis to study the effect of thalidomide on cancer cachexia and its possible mechanism of action by inhibition of TNF-α production. Our data demonstrated that thalidomide did not seem to straight interfere with cachexia and TNF-α serum levels in mice with squamous cells carcinoma. In humans, it was observed that thalidomide is capable of improving the subjective symptoms of cachexia, particularly by enhancement of the appetite and the sensation of well being (BRUERA et al., 1999). However, there was not evidence of the correlation between inhibition of TNF-α by thalidomide and cachexia. Finally, to our knowledge this is the first experimental study designed to investigate the role of thalidomide on cancer cachexia.

5 REFERENCES

AARESTRUP, F.M.; GONÇALVES DA COSTA, S.C.; SARNO, E.M. The effect of thalidomide on BCG-induced granulomas in mice. **Brasilian Journal of Medical and Biological Research**, Ribeirão Preto, v. 28, n. 10, p. 1069-76, 1995.

BALMAIN, A.; HARRIS, C.C. Carcinogenesis in mouse and human cells: parallels and paradoxes. **Carcinogenesis**, Oxford, v. 21, n. 3, p. 371-7, 2000.

BAUDITZ, J.; WEDEL, S.; LOCHS, H. Thalidomide reduces tumour necrosis factor α and interleukin 12 production in patients with chronic active Crohn's disease. **Gut**, London, v. 50, p. 196-200, 2002.

BOASBERG, P.D.; O'DAY, S.J.; WEISBERG, M. et al. Thalidomide induced cessation of weight loss and improved sleep in advanced cancer patients with cachexia. **Proceedings of the American Society of Clinical Oncology**, Alexandria, v. 19, p. 609, 2002.

BRUERA, E.; NEUMANN, M.; PITUSKIN, E.; CALDER, K.; BALL, G.; HANSON, J. Thalidomide in patients with cachexia due to terminal cancer: Preliminary report. **Annals of Oncology**, Viganello-Lugano**,** v. 10, p. 857-9, 1999.

CALABRESE, L.; FLEISCHER, A.B.Jr. Thalidomide: Current and potential clinical applications. **American Journal of Medicine**, Tucson, v. 108, p. 487-95, 2000.

DENG, L.; DING, W.; GRANSTEIN, R.D. Thalidomide Inhibits Tumor Necrosis Factor-α Production and Antigen Presentation by Langerhans Cells. **Journal of Investigative Dermatology**, Dallas, v. 121, p. 1060-5, 2003.

DUBOWSKI, A.; JOHNSTON, D.A.; RUPP, T., BELTRAN, L.; CONTI, C.J.; DIGIOVANNI, J. Papillomas at high risk for malignant progression arising both early and late during two-stage carcinogenesis in SENCAR mice. **Carcinogenesis**, Oxford*,* v. 19, n. 6, p. 1141-7, 1998.

ELEUTHERAKIS-PAPAIAKOVOU, V.; BAMIAS, A.; DIMOPOULOS, M.A. Thalidomide in cancer medicine. **Annals of Oncology**, Viganello-Lugano**,** v. 15, p. 1151-60, 2004.

FAVER, I.R.; GUERRA, S.G.; DANIEL, S.W.P.; EL-AZHARY, R. Thalidomide for dermatology: a review of clinical uses and adverse effects. **International Journal of Dermatology**, Malden*,* v. 44, p. 61-7, 2005.

FRANKS, M.E.; MACPHERSON, G.R.; FIGG, W.D. Thalidomide. **Lancet**, London, v. 363, p. 1802-11, 2004.

GERSON-CWILICH, R.; SERRANO-OLVERA, A.; VILLALOBOS-PRIETO, A. Talidomida en cáncer avanzado. Estudio fase I. **Cirurgia e Cirujanos**, Ciudad de Mexico, v. 69, p. 62-7, 2001.

GLICK, A.B.; YUSPA, S.H. Tissue homeostasis and the control of the neoplastic phenotype in epithelial cancers. **Seminars in Cancer Biology**, Amsterdan, v. 15, p. 75-83, 2005.

GOLDBERG, R.M.; LOPRINZI, C.L.; MAILIARD, J.A.; O'FALLON, J.R.; KROOK, J.E.; GHOSH, C. et al. Pentoxifylline for treatment of cancer anorexia and cachexia? A randomized, double-blind, placebo-controlled trial. **Journal of Clinical Oncology**, Alexandria, v. 13, n. 11, p. 2856-9, 1995.

GORDON, J.N.; TREBBLE, T.M.; ELLIS, R.D.; DUNCAN, H.D.; JOHNS, T.; GOGGIN, P.M. Thalidomide in the treatment of cancer cachexia: a randomised placebo controlled trial. **Gut**, London, v. 54, p. 540-5, 2005.

HASLETT, P.; HEMPSTEAD, M.; SEIDMAN, C.; DIAKUN, J.; VASQUEZ, D.; FREEDMAN, V.H. et al. The metabolic and immunologic effects of short-term thalidomide treatment of patients infected with the human immunodeficiency virus **AIDS Research and Human Retroviruses**, New Rochelle, v. 13, n. 12, p. 1047-54, 1997.

HIRST, G.L.; BALMAIN, A. Forty years of cancer modelling in the mouse. **European Journal of Cancer**, Edinburgh, v. 40, p. 1974-80, 2004.

KUMAR, S.; WITZIG, T.E.; RAJKUMAR, S.V. Thalidomide as an anti-cancer agent. **Journal of Cellular and Molecular Medicine**, Bucharest, v. 6, n. 2, p. 160-74, 2002.

LIN, J.H.; YANG, X.; BOSTROM, M.P.G. Thalidomide blocking of particle-induced TNF-α release in vitro. **Journal of Orthopaedic Association**, Tokio, v. 8, p. 79-83, 2003.

MOORE, R.J.; OWENS, D.M.; STAMP, G.; ARNOTT, C.,; BURKE, F.; EAST, N. et al. Mice deficient in tumor necrosis factor-α are resistant to skin carcinogenesis. **Nature Medicine**, New York, v. 5, n. 7, p. 828-31, 1999.

MOREIRA, A.L.; SAMPAIO, E.P.; ZMUIDZINAS, A.; FRINDT, P.; SMITH, K.A.; KAPLAN, G. Thalidomide Exerts Its Inhibitory Action on Tumor Necrosis Factor by Enhancing mRNA Degradation. **Journal of Experimental Medicine**, New York, v. 177, p. 1675-80, 1993.

OWENS, D.M.; WEI, S-J.C.; SMART, R.C. A multihit, multistage model for chemical carcinogenesis. **Carcinogenesis**, Oxford*,* v. 20, n. 9, p. 1837-44, 1999.

PARK, K-K; KIM, Y.S. Tumor associated proteins of rat skin tumor induced by 7,17-Dimethylbenz[a]anthracene. **Yonsei Medical Journal**, Seoul**,** v. 30, n. 3, p. 212-34, 1989.

PITOT, H.C.; DRAGAN, Y.P. Facts and theories concerning the mechanisms of carcinogenesis. **Federation of American Societies for Experimental Biology Journal**, Bethesda, v. 5, p. 2280-6, 1991.

RAJKUMAR, S.V. Thalidomide: Tragic Past and Promising Future. **Mayo Clinic Proceedings**, Rochester, v. 79, n. 7, p. 889-903, 2004.

RICHARDSON, P.; HIDESHIMA, T.; ANDERSON, K. Thalidomide: Emerging role in cancer medicine. **Annual Review of Medicine**, Palo Alto, v. 53, p. 629-57, 2002.

SAMPAIO, E.P.; SARNO, E.N.; GALILLY, R.; COHN, Z.A.; KAPLAN, G. Thalidomide Selectively Inhibits Tumor Necrosis Factor α Production by Stimulated Human Monocytes. **Journal of Experimental Medicine**, New York, v. 173, p. 699-703, 1991.

SCOTT, K.A.; MOORE, R.J.; ARNOTT, C.H.; EAST, N.; THOMPSON, R.G.; SCALLON, B.J. et al. An anti-tumor necrosis factor-alpha antibody inhibits the development of experimental skin tumors. **Molecular Cancer Therapeutics**, Philadelphia, v. 2, n. 5, p. 445-51, 2003.

SHARMA, R.; ANKER, A.D. Cytokines, apoptosis and cachexia: the potential for TNF-α antagonism. **International Journal of Cardiology**, Amsterdam, v. 85, p. 161-71, 2002.

SHERRY, B.A.; GELIN, J.; FONG, Y.; MARANO, M.; WEI, H.; CERAMI, A. et al. Anticachectin/tumor necrosis factor-**α** antibodies attenuate development of cachexia in tumor models. **Federation of American Societies for Experimental Biology Journal**, Bethesda, v. 3, p. 1956-62, 1989.

SLEIJFER, S.; KRUIT, W.H.J.; STOTER, G. Thalidomide in solid tumours: the resurrection of an old drug. **European Journal of Cancer**, Edinburgh, v. 40, p. 2377–82, 2004.

STOLER, A.B.; STENBACK, F.; BALMAIN, A. The Conversion of Mouse Skin Squamous Cell Carcinomas to Spindle Cell Carcinomas Is a Recessive Event. **Journal of Cell Biology**, New York, v. 122, n. 5, p. 1103-7, 1993.

STRASSMANN, G.; FONG, M.; KENNEY, J.S.; JACOB, C.O. Evidence for the Involvement of Interleukin 6 in Experimental Cancer Cachexia. **The Journal of Clinical Investigation**, Michigan, v. 89, p. 1681-4, 1992.

STROUD, M. Thalidomide and cancer cachexia: old problem, new hope? **Gut**, London, v. 54, p. 447-8, 2005.

SUGANUMA, M.; OKABE, S.; MARINO, M.W.; SAKAI, A.; SUEOKA, E.; FUJIKI, H. Essential Role of Tumor Necrosis Factor α (TNF-α) in Tumor Promotion as Revealed by TNF-α-deficient Mice. **Cancer Research**, Philadelphia, v. 59, p. 4516-8, 1999.

THOMAS, D.A.; KANTARJIAN, H.M. Current role of thalidomide in cancer treatment. **Current Opinion in Oncology**, London**,** v. 12, p. 564-73, 2000.

TISDALE, M.J. Biology of Cachexia. **Journal of the National Cancer Institute**, Bethesda, v. 89, n. 23, p. 1763-73, 1997.

TISDALE, M.J. Cancer cachexia. **Langenbecks Archive of Surgery**, Heidelberg, v. 389, p. 229-305, 2004.

TRACEY, K.J.; WEI, H.; MANOGUE, K.R.; FONG, Y.; HESSE, D.G.; NGUYEN, H.T. et al. Cachectin/Tumor Necrosis Factor induces cachexia, anemia, and inflammation. **Journal of Experimental Medicine**, New York, v. 167, p. 1211-27, 1988.

TRAMONTANA, J.M.; UTAIPAT, U.; MOLLOY, A.; AKARASEWI, P. et al. Thalidomide treatment reduces tumor necrosis factor alpha production and enhances weight gain in patients with pulmonary tuberculosis. **Molecular Medicine**, Manhasset, v. 1, n. 4, p. 384-97, 1995.

YONEDA, T.; ALSINA, M.A.; CHAVEZ, J.B.; BONEWALD, L.; NISHIMURA, R.; MUNDY, G.R. Evidence That Tumor Necrosis Factor Plays a Pathogenetic Role in the Paraneoplastic Syndromes of Cachexia, Hypercalcemia, and Leukocytosis in a Human Tumor in Nude Mice. **The Journal of Clinical Investigation**, Michigan, v. 87, p. 977-85, 1991.

YUSPA, S.H. The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis. **Journal of Dermatological Science**, Nagoya, v. 17, p. 1-7, 1998.

WINES, N.Y.; COOPER, A.J.; WINES, M.P. Thalidomide in dermatology. **Australasian Journal of Dermatology**, Richmond v. 45, p. 229-40, 2002.

WOODWORTH, C.D.; MICHAEL, E.; SMITH, L.; VIJAYACHANDRA, K.; GLICK, A.; HENNINGS H.; YUSPA S.H. Strain-dependent differences in malignant conversion of mouse skin tumors is an inherent property of the epidermal keratinocyte. **Carcinogenesis**, Oxford, v. 2, n. 9, p. 1771-8, 2004.

ZOUMPOURLIS, V.; SOLAKIDI, S.; PAPATHOMA, A.; PAPAEVANGELIOU, D. Alterations in signal transduction pathways implicated in tumour progression during multistage mouse skin carcinogenesis. **Carcinogenesis**, Oxford, v. 24, n. 7, p. 1159-65, 2003.