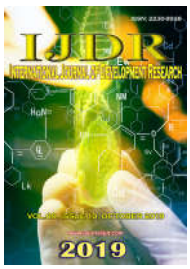
ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 09, Issue, 10, pp. 30942-30945, October, 2019



RESEARCH ARTICLE OPEN ACCESS

COMPARISON OF EXPERIMENTAL ORAL MELANOMA IN MICE AND ORAL MELANOMA IN DOGS

***[1]Louise Bach Kmetiuk, [2]Renato van Wilpe Bach, [2]Leandro Cavalcante Lipinski, [3]Andrigo Barboza de Nardi, [3]Rafaela Bortolotti Viéra, [4]Gilson Cesar Nobre Franco, [4]Katia Sabrina Palludo and [4]Giovani Marino Fávero**

[1]Department of Cellular and Molecular Biology, Federal University of Paraná, Curitiba, PR, 81531-900, Brazil
[2]Department of Medicine, State University of Ponta Grossa, PR, 84030-900, Brazil
[3]Department of Veterinary Clinics and Surgery; São Paulo State University, SP, Jaboticabal, 14884-900, Brazil
[4]Department of Odontology, State University of Ponta Grossa, PR, 84030-900, Brazil
[5]Department of General Biology, State University of Ponta Grossa, PR, 84030-900, Brazil

ARTICLE INFO

Article History:
Received 27th July, 2019
Received in revised form
14th August, 2019
Accepted 19th September, 2019
Published online 30th October, 2019

Key Words:

Oral melanoma, Canine melanoma, Murine melanoma.

****Corresponding author: Louise Bach Kmetiuk***

ABSTRACT

Canine oral melanoma is a malignant tumor, with no reports onexperimentalmodel to date. Accordingly, the aim of this study was development the oral melanoma in mice and comparewith naturally occurring canine oral melanoma. A total of 30 C57BL/6J male mice received tumor induction, with euthanasia at 7th, 14th and 21thpost-operative days, and hemimandible resection. Macroscopic analyses have been performed in 18/30 (60.0%) mice. Morphological aspects and histopathologicalcharacteristics of the murine has shown similarities with canine oral melanoma. Thus, the murine oral melanoma may be useful, viable and reproducible alternative on preclinical study model for dogs.

Citation: Louise Bach Kmetiuk, Renato van Wilpe Bach, Leandro Cavalcante Lipinski *et al.* 2019. "Comparison of experimental oral melanoma in mice and oral melanoma in dogs", *International Journal of Development Research*, 09, (10), 30942-30945.

INTRODUCTION

Melanoma has been the maincanine oral malignant tumor, associated with aggressive progression and resistance to proposed therapies (Ramos-Vara *et al*., 2000; Smith*et al*, 2002; Bergman, 2007; Poorman *et al*., 2014). Gum has been the principal tumor site (Gioso, 2005). Oral melanoma can be delimited in oral mucosa, with benign behavior and expressive amount of melanin (Gioso, 2005). Malignancyhas been associated with unencapsulated tumor, regional infiltration, tooth exfoliation and metastatic potential, especially in regional lymph nodes and lung (Smith, 2005). Models of melanoma study have been used for promoting preclinical approaches of disease progression (Santiago-Walker *et al*., 2009). The B16 murine melanoma has been the second most commonly model for skin disease (Fávero *et al*., 2010). The cell line was first isolated from spontaneously melanoma in C57BL/6J mice, with varieties to F1 from F10, according to the metastatic capacity (Fidler, 1973). Intensemelanin pigmentation, tumor accelerated growth *in vivo* and high metastatic capacity, with death resulting from tumor progression in an average of 15 to 30 days have been relatedwith B16F10 cell line (Fidler and Nicholson, 1976). The high casuistic, accelerated progression, as well as the absence of considerable therapeutic of canine oral melanoma promoted the requirement for preclinical trials model.Accordingly, the aim of this study was development the oral melanoma in mice and comparewith naturally occurring canine oral melanoma.

MATERIALS AND METHODS

In vivo experiments were conducted in male C57BL/6J mice, housed at 22 ± 2°C under a 12: 12 hourslight: dark cycle with *ad libitum* food and water access. The animals were acclimatized to the laboratory for at least 24 hours before testing and were used only once. All animal handling and experimental procedures were conducted with prior approval

Table 1.

Group	POD	n (Inicial)	n(Final)		Mean	Median	Standard deviation	Standard error
	7º	5	4		0.0287	0.0322	0.0212	0.0106
1 ($5x10^4$)	14º	5	3	Tumor volume mm^3	1.2626	1.2584	0.1415	0.0817
	21º	5	2		2.2838	2.2838	1.5355	1.0858
	7º	5	5	Tumor volume mm^3	0.0009	0.0005	0.0006	0.0003
2 ($1x10^4$)	14º	5	5		0.0120	0.0096	0.0129	0.0065
	21º	5	2		0.4836	0.4836	0.4192	0.2964
N (Total)		30	21					

of the ethics committee on animal use of the State University of Ponta Grossa Ethics Committee (numbers: 8398/2015, 674/2016). B16F10 mice melanoma cell linehas been regularly cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum (FBS), at 37°C in a humidified atmosphere containing 5% CO_2. Cell viability and quantification were also obtained by the method of Trypan Blue exclusion (Invitrogen, Carlsbad, CA), by direct counting in a hemocytometer. Initially, C57BL/6J mice (*N*=30) have been anesthetized with intraperitoneal ketamine/xylazine at a dose of 100/10 mg/kg body weight. In sequence, two different concentrations of B16F10 mice melanoma cell line were injected into the vestibular side of ventral gum. For the tumor induction with B16F10 mice melanoma cell line, the animals were randomized distributed in two groups, as follows: group 1 (n=15) that have been injected with $1x10^4$ cells and group 2 (n=15) that have been injected with $5x10^4$ cells.

Animals have been euthanized by cervical dislocation(Fávero *et al*., 2010) at the seventh, fourteenth and twenty-first post-operative days (POD).The tumor volume in mm^3has been obtained using the formula suggested by Novak *et al*., 2003:
Mice hemimandibles have been resected for mensuration and histological analysis. Tissue sampleswere fixed and stained with hematoxylin-eosin.Fixed tissues in paraffin blocks of 19 canine oral melanoma caseshave been received from Veterinary Hospital of São Paulo State University, Jaboticabal-SP,for histopathological comparative analysis.For descriptive and morphometric histological analyzes, an Olympus Bx41 microscope coupled to a digital DP-Controler capture system has been used to obtain images corresponding to 12 visual fields *per*slide, when possible.For descriptive histology, have been noted: tumor invasion in adjacent tissues; presence of melanin granules; cell type (rounded, elongated, or both-mixed cells); characteristics of the cytoplasm, nucleus and presence of inflammatory infiltrate. Image J software (NHI Program) has been used for morphometric analysis. The vascularization and necrosis areas have been quantified in 12 fields *per* slide and comparing to the total field area. The results were statistically analyzed by non-parametric statistical tests, and expressed as mean ± standard deviation. A *p* value lower than 0.05 has been considered significant. All tests have been performed using BioStat 5.3.

RESULTS

A total of 18/30 (60.0%) mice have been developed visibly tumor and 3/30 (10%) not developed (Group 2). A total of 9/30 (30.0%) animals died before the post-operative days determined to euthanasia, 6/9 (66.6%) from group 1 and 3/9 (33.3%) from group 2 (Table 1). Non-Spearman's parametric testshowed positive correlation means of the tumor volume and the time of development (rs = 1, p <0.0001) (p<0.0001).

Tumor formation (macula or mass), staining (red, brown or black), regularity of margins (regular or irregular) and ulceration (presence or absence) have been noted (Supporting information 1). The nominal qualitative variables (type of formation, staining, regularity margins) and continuous quantitative (tumor volume) have been transformed into ordinal qualitative, in order to perform qualitative analyzes. The Kruskal-Wallis test has been used with Dunn's ad hoc test. The Dunn post-test showed a difference between: formation type and ulceration (p<0.05); tumor volume and staining (p<0.05); tumor volume and ulceration (p<0.05); staining and ulceration (p<0.05); regularity of borders and ulceration (p<0.05). Presence of correlation has been tested by Spearman Non-Parametric Test in variables that obtained a statistically significant difference. Positive correlation has been observed between the type of tumor formation (macula or mass) and volume (rs = 0.6038; p<0.037). The presence of ulceration has been associated with mass in all cases. Positive correlation has been observed between tumor volume and staining (red, brown or black). Black coloration has been associated with mass in all cases, and red or brown staining with macula. Occurrence of ulceration may associated with vertical tumor growth (rs = 0.4889; p<0.0245) and black staining (rs=0.7002; p<0.0004). Border regularity and ulceration have been demonstrated positive correlation (rs = 0.3491, p = 0.1207). The histopathological analysis of murine oral melanoma at 14º post-operatory day for Group 1 showed the presence of proliferating of melanocytes and melanin granules in muscle tissue (Fig. 1; Fig 2).

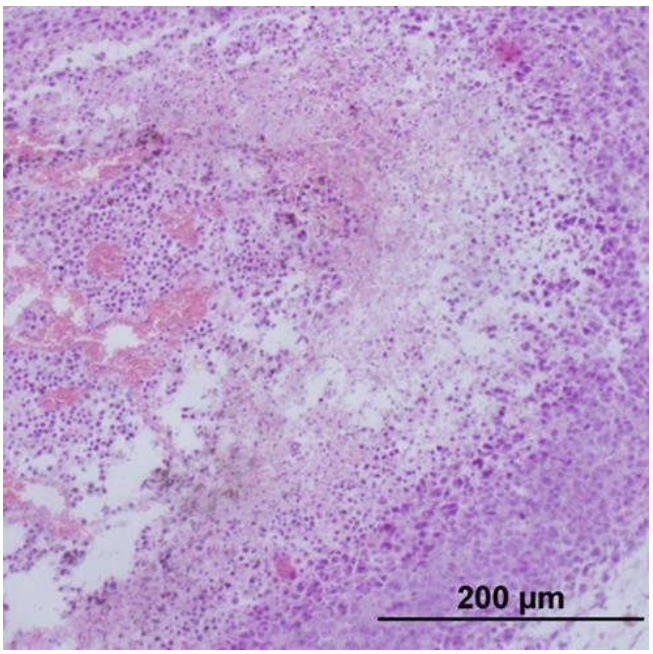


Fig. 1. Photomicrographs of histological sections of tumor formation. Melanin granules, angiogenesis and loss of tissue integrity have been observed in murine oral melanoma

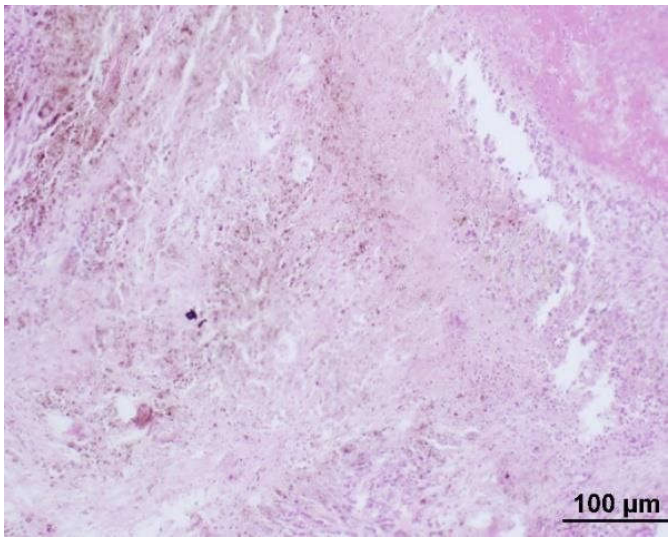


Fig. 2. Photomicrographs of histological sections of tumor formation. Melanin granules, angiogenesis and loss of tissue integrity have been observed in canine oral melanoma

The reduction of nuclear elements distinctness has been evidenced the coagulation necrosis area. Necrosis areas mean for the 12 fields totalized 41.72%. Relative loss of integrity, regular delineation of blood vessels andangiogenesis mean of 0.5%have been observed. The 14 post-operatory day of group 2 has shown mixed cell aspect, melanin granules, intense lymphocytic inflammatory infiltrate and proliferating melanocytes. Necrotic areas were 14.2%. The blood vessels have been showed the loss of integrity of walls, and the vascularization calculated in 1.2%average. The cases of canine oral melanoma have been histologically analyzed. Similarly to murine oral melanoma, melanin granules, proliferating melanocytes with diffusely invasion in nearby tissues and inflammatory infiltrate have been present. Large nucleus and cytoplasm in mixed cells have been observed. From the 12 fields analyzed for these cases, necrosis average has been of 89.2%, and vascularization ratesof 1.1%.

DISCUSSION

To the authors knowledge, this is the first-experimental murine oral melanoma development as preclinical model of canine oral melanoma. C57BL/6J received different cells concentrations for tumor induction. Tumor aggressiveness may be influenced by cell concentration and uniformity of cell line preparations cell agglomeration (Fidler, 1973). Thus, the high mortality rate and tumor progression of group 1 may be related with cell concentration of tumor induction. The qualitative analysis of the macroscopic characteristics according to cell concentration and post-operative day may suggested disparity of tumor growth behavior. The positive correlation between ulceration and type of formation, tumor volume and staining have been observed. Ulceration has been associated with black staining mass in all cases. These characteristics agreementwith a previous studies of canine oral melanoma (Smith, 2005; Bergman, 2012). Initial radial growth has been observed in both, group 2 and canine oral melanoma, and demonstrated in the comparison of tumor volumes means *versus* post-operation days means. Furthermore, the staining, border regularity and type of formation corroborating with previous studies of canine oral melanoma (Hernandez *et al*., 2018;Gioso, 2005; Poorman *et al*., 2014; Requicha *et al*., 2015).

Group 1 of murine oral melanoma has shown high aggressive than experimental model of cutaneous melanoma (Fidler, 1973), with 1/5 (20.0%) death earlier seven post-operative days and 2/5(40.0%) deaths before fourteen post-operative days.The period of primary tumor survival has been required for model establishing, which makes possible to relate tumor progression and therapeutic agent (Santiago-Walker *et al*, 2009). The extensive resection of experimental murine oral melanoma tumor formation may be allowed more comprehensive histopathological analysis of *in situ* or radial involvement if compared with dogs tissue samples, frequently withincomplete resection due to anatomical location (Simpson *et al*., 2013). Comparative histopathological analysis have shown invasion of adjacent tissues, angiogenesis capacity and necrotic areas in both species and may be denoted rapid vertical growth (Spangler and Kass, 2006; Pereira *et al*., 2008). Neoplasic cells herein have been a mixed appearance in both dogs and mice, as well as the presence of elongated and rounded cells in bundles, with evident nucleus. Finally, quantitative and analyzes of tumor volume have shown disparity tumor growth behavior, related to cell concentration and the post-operative day. Positive correlations between tumor volume and ulceration, staining and ulceration and type of formation and ulceration may be associated with tumor development and progression. Staining, regularity, type of formation, as well as histopathological characteristics occurred in agreement with that described in the literature for canine oral melanoma. To the authors knowledge, the present study has been first-experimental murine oral melanoma. In addition, the induction of oral melanoma in C57BL/6J mice for purposes of preclinical study model for canine oral melanoma showed useful, viable and reproducible alternative.

Acknowledgements: Authors are kindly thankful to Dr Fábio André dos Santos and Dr Iriane Eger.

Competing interests: The authors declare no competing interests.

Funding: This work has been supported by the Higher Education Improvement Coordination (CAPES-DS), Brazil.

REFERENCES

Bergman, J.P. Melanoma Review. 2012. Updated September.

Bergman, P.J. 2007. Canine oral melanoma. *Clinical Techniques in Small Practice.* 22, pp. 55-60.

Favero, G.M, Otuki, M. F., Oliveira, K. A., Bohatch Jr, M. S., Borelli, P., Barros, F. E., Maria, D. A., Fernandes, D. and Bydlowski, S. P. 2010. Simvastatin impairs murinemelanoma growth. Lipids Health Disease.9, pp. 142.

Fidler, I.J. 1973. Selection of Successive Tumour Lines for Metastasis. Nature.242, pp. 148-149.

Fidler, I.J. and Nicolson, G.L. 1976. Organ selectivity for implantation survival and growth of B16 melanoma variant tumor lines. Journal of the National Cancer Institute. 57, pp. 1199-202.

Gioso, M.A. 2005. Neoplasias de la cavidad oral. II Congreso Nacional de la Sociedad Española de Odontología, Cirugía Maxilofacial Veterinarias y Experimental (Livro de Resumos). Madrid, Espanha.

Hernandez, B., Adissu, H.A., Wei, B.R., Michael, H.T., Merlino, G. and Simpson, R.M. 2018. Naturally Occurring Canine Melanoma as a Predictive Comparative Oncology Model for Human Mucosal and Other Triple Wild-Type Melanomas. *International Journal of Molecular Sciences.* 19(2), pp. 394.

Novak, E.M., Metzger, M., Chammas, R., da Costa, M., Dantas, K., Manabe, C., Pires, J., de Oliveira, A.C. and Bydlowski, S.P. 2003. Downregulation of TNF-alpha and VEGF expression by Sp1 decoy oligodeoxynucleotides in mouse melanoma tumor. Gene Therapy.10, pp. 1992-1997.

Poorman, K., Borst, L., Moroff, S., Roy, S., Labelle, P., Motsinger-Reif, A. and Breen, M. 2015. Comparative cytogenetic characterization of primary canine melanocytic lesions using array CGH and fluorescence in situ hybridization. *Chromosome Research.* 23, pp. 2.

Ramos-Vara, J.A., Beissenherz, M.E., Miller, M.A., Johnson, G.C., Pace, L.W., Fard, A. and Kottler, S.J. 2000. Retrospective study of 338 canine oral melanomas with clinical, histologic, and immunohisto-chemical review of 129 cases. *Veterinary Pathology*. 37, pp. 597-608.

Requicha, J.F., Pires, M.A., Albuquerque, C.M. and Viegas, C.A. 2015. Canine oral cavity neoplasias - Brief review. *Revista Brasileira de Medicina Veterinária*. 37(1), pp. 41-46.

Santiago-Walker, A., Li,L., Haass, N.K. andHerlyn, M. 2009. Melanocytes: From morphology to application. Skin Pharmacol. Physiology. 22, pp. 114-121.

Simpson, R.M. 2013. Sporadic naturally occurring melanoma in dogs as a preclinical model for human melanoma. *Pigment Cell Melanoma Res.* 27(1), pp. 37-47.

Smith, M.M. 2005. Oral and salivary gland disorders. Ettinger S.J. & Feldman E.C. (Eds). *Textbook of Veterinary Internal Medicine.* 2(6),pp. 1290-1296.

Smith, S.H., Goldschmidt, M.H., McManus, P.M. 2002. A comparative review of melanocytic neoplasms. Veterinary Pathology.39, pp. 651-678.

van der Weyden, L., Patton, E.E., Wood, G.A., Foote, A.K., Brenn, T., Arends, M.J. and Adams, D.J. 2015. Cross-species models of human melanoma. *The Journal of Pathology.* 238(2), pp. 152-165

****