

CD8⁺ T lymphocytes infiltrate in mammary tumours chemically-induced in female rats: what is the influence of ketotifen administration?

Oliveira, PA^{1,2}, Medeiros, M³, Branco, C^{4,5}, Faustino-Rocha, AI^{1,6,7}

¹ Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Inov4Agro, Vila Real, Portugal; ² Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; ³ Department of Biology, School of Sciences and Technology, University of Évora, Évora, Portugal; ⁴ Department of Veterinary Sciences, School of Sciences and Technology, University of Évora, Évora, Portugal; ⁵ Mediterranean Institute for Agriculture, Environment and Development (MED), Évora, Portugal; ⁶ Department of Zootechnics, School of Sciences and Technology, University of Évora, Évora, Portugal; ⁷ Comprehensive Health Research Center (CHRC), Évora, Portugal

Background: Mammary cancer is one of the most frequent cancers among women. Neoplasia are complex masses composed of both neoplastic and non-neoplastic cells, like vascular and lymphatic endothelial cells, adipocytes, mesenchymal cells, fibroblasts, myeloid and inflammatory cells (lymphocytes, neutrophils, eosinophils, macrophages, and mast cells). This work aimed to evaluate the effects of ketotifen on the infiltrate of CD8⁺ T lymphocytes in mammary tumours chemically-induced in female rats.

Material and methods: All experiments were performed in accordance with the legislation on the protection of animals used for scientific purposes. The experiments were approved by the Portuguese Competent Authority (no.008961) and University Ethics Committee (CE_12-2013). Thirty-four female Sprague-Dawley rats were randomly assigned to five experimental groups. At seven weeks of age, mammary tumours' development was induced in animals from groups I, II, III (n=10+10+10) by a single intraperitoneal injection of the carcinogen *N*-methyl-*N*-nitrosourea (MNU). Animals from groups IV and V were injected with saline. Groups II and IV (n=2) were treated with ketotifen in drinking water (1 mg/kg/day, 7 days/week) immediately after MNU administration for 18 weeks, while animals from group III received the ketotifen only after the development of the first mammary tumour. Groups I and V (n=2) received only water. Animals were sacrificed at 25 weeks of age by an overdose of ketamine and xylazine, followed by an exsanguination by cardiac puncture. Mammary tumors were collected and immersed in 10% buffered formalin. The infiltrate of CD8⁺ T lymphocytes was assessed by immunohistochemistry using the antibody anti-CD8 (ab33786; Abcam),

at a dilution of 1:250, overnight. The immunoexpression was evaluated manually, counting the number of positive cells in five random fields, at a magnification of 400x. Data was statistically analysed using Statistical Package for the Social Sciences (SPSS).

Results: Animals from groups IV and V did not develop any mammary tumor. The immunoexpression of anti-CD8 was evaluated in 56 tumours (19 from group I, 19 from group II and 18 from group III). The antibody presented a cytoplasmatic immunoexpression in all mammary tumours. The mean number of immunopositive cells was 21.75 ± 1.74 in group I, 21.75 ± 1.74 in group II and 22.75 ± 2.01 in group III. No differences were observed among groups ($p > 0.05$).

Conclusions: Apparently, the ketotifen administration did not modulate the infiltrate of CD8+ T lymphocytes in mammary tumours chemically-induced in female rats. Further studies addressing the effects of different concentrations of ketotifen are warranted.

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