**INTRODUCTION**

The spleen is a site for storage and rapid deployment of monocytes; splenic monocytes have been identified as a resource that the body exploits to regulate inflammation and anticancer immunological vigilance (Science 2009 325:1212-6).

We study the role of macrophages in human cancer since 1990, when we characterised the signalling transaction pathway of the human receptor for Macrophage-Colony Stimulating Factor (c-fms) (Proc Natl Acad Sci USA 1990; 87, pp. 5613-5617, August 1990).

In the present study, a well characterised stimulator of macrophages deriving from vitamin D binding protein (DBP-MAF/GeMaF), was conjugated with oleic acid (OA), a known anti-cancer agent as well as a protein absorption enhancer. Here we demonstrate that the dramatic effects of OA-GeMaF in cancer patients (Am J Immunol 10(1): 23-32, 2014) are mediated by nitric oxide (NO) produced by activated macrophages.

This identifies a triad of molecular elements, OA, GeMaF and NO, each one endowed with individual anti-cancer properties, that show synergistic effects and might open the way to a much improved cancer treatment strategy devoid of harmful side effects.

**RESULTS**


Considering the impressive effects of NO in pre-clinical models of cancer where it slows tumour growth and enhances the efficacy of both chemotherapy and radiotherapy (Cancer Chemother Pharmaco 67(6): 1211-1224, 2010), we believe that OA-GeMaF stimulated the release of NO from macrophages in vivo.

To this end, we chose to study the variation of the splenic blood flow, following the administration of OA-GeMaF.

According to our hypothesis, OA-GeMaF would stimulate the production of NO by tissue macrophages at the site of injection, or by alveolar macrophages when administered by nebulisation.

OA-GeMaF would then be absorbed into the bloodstream and it would eventually stimulate macrophages in other areas of the body. Considering the huge amount of macrophages residing in the spleen, we would expect a sustained release of NO by activated macrophages in this organ, with a concomitant increase of blood flow that we could measure by echo-colour-doppler (Esaote).

Activation of tissue, alveolar and splenic macrophages by OA-GeMaF administration with sustained release of NO, was associated with significant decrease in tumour volume in all cases where the tumour, metastases or lymphnodes could be measured by ultrasonographic techniques.

On average, we observed a decrease of tumour volume of about 25% in one week (Am J Immunol 10(1): 23-32, 2014).

These results are consistent with the hypothesis that OA, GeMaF and NO, molecules endowed with individual anti-cancer properties targeting different aspects of neoplastic growths, indicate a synergistic effect when administered in such a way to exploit their physiological characteristics.

Among the cases observed at the Immuno Biotech Treatment Centre, we present the cases of two metastasised breast cancer patients, for whom the integrative immunotherapy based on OA-GeMaF was remarkably effective.

**CONCLUSIONS**

The observations presented in this study, together with the recent results of molecular modelling (Nutrients 5(7): 2577-2589, 2013), demonstrate that OA, GeMaF and NO, molecules endowed with individual anti-cancer properties and in properly combined and spectrally delivered doses, can provide cancer patients with significant effects on immune system stimulation and tumour volume reduction.

These observations have the potential for revolutionising the field of cancer treatment since it appears that the proposed anti-cancer strategies are entirely devoid of any recorded harmful side effects.

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