An In Vivo Study on the Relationship Between Resveratrol and FasL (CD95L)

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ABSTRACT

Resveratrol is a phytoalexin found naturally in spermatophytes like grapes, peanuts and pines and had been shown to possess chemotherapeutic properties. Although the exact mechanisms of its chemotherapeutic actions had remained elusive, studies had isolated its role in triggering the Fas-FasL dependent apoptosis in tumor cells. This project had sought to establish a possible relationship between FasL and resveratrol in vivo with a lymphoproliferative (lpr) mouse model. The results had shown that the treatment with resveratrol in the lpr mice had induced an upregulation of FasL in their tumor cells. However, the results had been marred by a similar observation in the vehicle control, rendering it inconclusive.

INTRODUCTION

Resveratrol (\textit{trans}-3,4\textquotesingle,5-trihydroxystilbene) is a polyphenolic phytoalexin found naturally in spermatophytes like grapes, nuts and pines. The molecule had exhibited a wide range of biological effects. Its anti-oxidant, anti-platelet aggregation and anti-inflammatory effects had been proposed to impede atherosclerotic lesions and could be responsible for the cardio-protective observations made in the so-called \textit{French Paradox}. Also, it had displayed its anti-carcinogenic effects and was suggested as a potent cancer chemopreventive agent with a reduction in tumor incidence (Clifford et al, 1996). These effects of the resveratrol had been proposed to attribute to its suppression of the tumor necrosis factor (TNF)-induced activation of nuclear transcription factor NF-\(\kappa\)B and activator protein-1 (AP-1) (Manna et al, 2000).

Besides the above biological effects, the chemotherapeutic role of resveratrol had also been an area of intensive research. It had been shown that resveratrol could decrease tumor growth (Carbo et al, 1999) and in the earlier studies conducted by our laboratory, we had attributed the chemotherapeutic effects of resveratrol to an increased Fas-FasL interaction, which subsequently triggered an increase in apoptosis of the tumor cells; the Fas-FasL interaction would have engaged capase 9 and downstream capase 3 in the apoptotic event. The treatment with resveratrol had observed an upregulation of FasL on HL60 human leukemia cell line and T47D breast carcinoma cells in vitro, which had correlated with resveratrol-induced cell cytotoxicity (Clement et al,1998; Pervaiz, 2000).

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Then in order to take physiological conditions into consideration, we had adopted an \textit{in vivo} model. Our murine skin carcinogenesis model had revealed an \textit{in vivo} triggering of apoptosis by resveratrol (Pervaiz, 2000). And because of the positive results from the resveratrol-induced apoptosis of the \textit{in vivo} model, it had been the aim of this project to attempt to see if we could replicate the relationship between FasL and resveratrol observed in the \textit{in vitro} model to support that the resveratrol could have manifest its chemotherapeutic effects via the Fas-FasL pathway under physiological conditions.

**MATERIALS AND METHODS**

**Treatment**

6 lpr mice of strain C3-MRL and each weighing 25-35g had been used for the experiment. The mice were divided into 3 groups of two for control, vehicle control and resveratrol-treated. The treated mice were weighted and injected intra-peritoneally with resveratrol dissolved in dimethylsulfoxide (DMSO): PBS [1:20] over a period of 21 days at 1-day interval. The amount of resveratrol injected was done at 1mg/kg b.w. Similar injection were done on the vehicle control but only with DMSO:PBS.

**Perfusion and Extraction of Tumors**

At the end of the 21-day treatment, the mice were put to sleep with 7% choral hydrate and subsequently perfused with Ringer’s solution, followed by 4% paraformaldehyde for fixation. The tumors were then removed from the mice and soaked in 4% paraformaldehyde at 4°C overnight for further fixation.

**Sectioning**

The fixed tumors were transferred from paraformaldehyde and immersed in 15% sucrose overnight for dehydration before sectioning. The tumors were sectioned at 16\(\mu\)m using cryostat and the sections were then mounted onto gelatinized slides that were prepared beforehand.

**Immunohistochemistry**

Immunohistochemistry was performed on the sections over 2 days. The control sections were stained for FasL and 2\textsuperscript{nd} antibody while the vehicle control and resveratrol-treated sections were stained for FasL only. The immunohistochemistry technique had employed the use of 3,3-diaminobenzidine (DAB) for the staining.
RESULTS

From the results of the DAB staining, we could see that there had been a visible and significant increase in the number of FasL (upregulation) in the tumors of resveratrol-treated mice compared to the controls’ (Fig. 1 and 2). Further, FasL could often be found throughout a section from the treated cells while in contrast, FasL could usually only be located in a small area of a section from the control cells (not shown).

Fig 1 shows an upregulation of FasL in resveratrol-treated cells while Fig 2 shows the presence of only a handful of FasL in the controls. The sections were taken at 20x and they represent a typical section from each group.

However, although there had been an observed upregulation of FasL in the resveratrol-treated cells from Fig. 1 and 2, further analysis with the vehicle control had showed similar upregulation of FasL (Fig. 3) compared to the treated cells, and which also could be found throughout a section from the vehicle control cells.

Fig. 3. A typical section from vehicle control cells at 20x.
The DAB staining on the control sections for 2nd antibody had showed little background staining and thus it would not have interfered with our interpretation of results.

DISCUSSION

The upregulation of FasL in the vehicle control cells could have been an indication of a high concentration of DMSO at 20x dilution. Because DMSO is toxic at too high a concentration, it could have initiated the apoptotic pathway of the lymphocytes (tumor) and caused an upregulation of FasL. The concentration of the vehicle was from the experiment by Carbo et al (1999) who had shown a decrease of tumor growth with the administration of resveratrol. In their experiment, Carbo et al had revealed a significant difference in total cell number for their treated and vehicle tumor cells. While their results could not be disputed at the moment, based on our findings, it could be postulated that resveratrol and the vehicle had an additive effect in triggering apoptosis of the tumor cells. But I would also like to point out that Carbo et al had not have a tumor control, which might have presented a huge disparity in the number of FasL between that and the vehicle control and treated cells, as we had done and shown. However, this had only been a speculation. Further, the rats that had been used by Carbo et al were not immunodeficient like ours (lpr mice) and thus could have provided a better resistance to the DMSO.

Nevertheless, because of the similar upregulation of FasL in our vehicle controls as compared to our treated cells, we could not conclude that resveratrol had caused an upregulation of FasL (with our staining results). To resolve the problem, it would be necessary to either adopt a much greater dilution of the vehicle but yet ensuring the solubility of resveratrol, or use an alternative solvent.

REFERENCES


