

RAPID COMMUNICATION

## The Effect of Quercetin on Light-Induced Cytotoxicity of Hypericin

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Received January 11, 2001

Accepted February 16, 2001

### Summary

Protective effect of quercetin, a natural antioxidant compound, on hypericin-induced cytotoxicity was studied in human promyelocytic leukemia cells (HL-60). Hypericin ( $10^{-5}$  mol.l<sup>-1</sup>) alone significantly decreased cell survival to 21 % that found in the controls, whereas in combination with quercetin ( $10^{-5}$  mol.l<sup>-1</sup>) this decrease was diminished to 46 %. Lower concentrations of quercetin had no protective effect. These findings indicate that oxygen radicals can play an important role in hypericin-induced phototoxic effects.

### Key words

Hypericin • Quercetin • Cell line • Oxygen radicals • Cytotoxic activity

### Introduction

Hypericin is a photosensitizing pigment abundantly found in wildflowers from the family *Hypericum* (*H. perforatum*, St. John's worts). The recent interest in hypericin was spawned by the discovery that it possesses extremely high toxicity towards certain viruses and tumors. Hypericin-induced photosensitization has been extensively investigated by many researchers. Some of them suggest that hypericin's cytotoxic effect is due to the production of bioreactive oxygen species, mainly of singlet oxygen (type II photosensitization) or semiquinone radicals (type I photosensitization) (Diwu and Lown 1992, 1994).

However, it was found several years ago that other mechanisms may also be involved in hypericin

phototoxicity. It was suggested that hypericin is able to produce a photogenerated pH drop *via* an intramolecular proton transfer, which is likely to precede solvent acidification (Carpenter *et al.* 1994). This hypothesis was confirmed later by many other investigators (Sureau *et al.* 1996, Mirossay *et al.* 1999, Miroššay *et al.* 1999, Das *et al.* 1999).

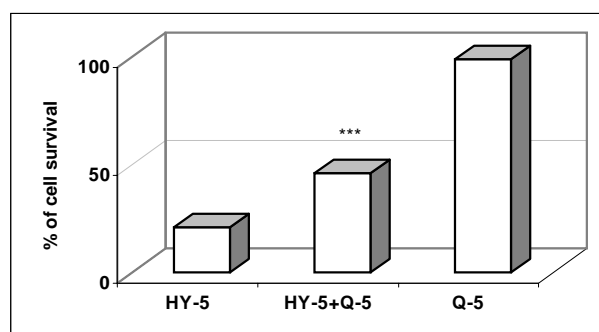
The aim of the present study was to determine a possible effect of quercetin, a naturally occurring antioxidant compound, on hypericin-induced phototoxicity in HL-60 cells and consequently to support the hypothesis about the role of singlet oxygen in the biological activity of hypericin.

The HL-60 promyelocytic cell line was kindly provided by Dr. M. Hajdúch (Olomouc, Czech Republic). This cell line was maintained in Dulbecco's MEM (DMEM) containing 10 % FCS, glutamine and penicillin/

streptomycine. Hypericin (Carl-Roth, Germany) and quercetin (Sigma, USA) were dissolved in dimethylsulfoxide (DMSO) and diluted to a final concentration (0.25 %) with DMEM. A power-controlled low-intensity halogen lamp was used as a light source in the experiment. The cell cultures were illuminated with a total light dose of 4 J/cm<sup>2</sup>. For assessing the cytotoxic effect of the tested agents the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method was used in all experiments (Mirossay *et al.* 1999). Statistical analysis of the result was carried out by Student's t-test. The results with  $p < 0.001$  were taken as statistically significant.

Since the cytotoxic effect of hypericin requires illumination, the experiments were performed under light conditions. The maximal cytotoxic effect of hypericin was observed at  $10^{-5}$  mol.l<sup>-1</sup> concentration. Using this concentration the percentage of surviving cells reached about 21 %. A lower hypericin concentration,  $10^{-6}$  mol.l<sup>-1</sup>, also exhibited a significant cytotoxic effect, but the survival of cells was about 86 % (data not shown). There was no significant cytotoxic effect of quercetin at the concentration  $10^{-5}$  mol.l<sup>-1</sup>. Moreover, quercetin alone did not exert any cytotoxic effects when tested at other concentrations. The combination of hypericin and quercetin can be seen in Figure 1. Quercetin at a concentration  $10^{-5}$  mol.l<sup>-1</sup> had a significant protective effect against the cytotoxicity of hypericin. Cell survival for this combination was 46 % compared to 21 % for hypericin alone. No significant protective effect was observed for lower concentrations of quercetin ( $10^{-6}$  mol.l<sup>-1</sup>) and hypericin ( $10^{-6}$  mol.l<sup>-1</sup>) as well as for their combination (data not shown).

Quercetin, one of the most abundant of flavonoids, possesses many biological effects, and it is assumed that several of them are due to its antioxidant activity (Korkina and Afanasev, 1997, da Silva *et al.* 1998, Terao 1999, Erden Inal and Kahrman 2000, Mojžišová and Kuchta 2001). The antioxidant action of quercetin is expected to be similar to that of other polyphenols. They are able to act as antioxidants by virtue of the hydrogen-donating capacity of their phenolic groups (Morel *et al.* 1993). The antiradical property of flavonoids is directed towards many free radicals such as the superoxide, hydroxyl radical as well as peroxy and alkoxy radicals (Husain *et al.* 1987). Later, it was found that quercetin is also a strong singlet oxygen quencher (Tournaire *et al.* 1993).



**Fig. 1.** The cytotoxic effect of hypericin was observed at  $10^{-5}$  mol.l<sup>-1</sup> concentration (HY-5) with cell survival reached about 21 %. Cell survival for combination with quercetin (HY-5+Q-5) was 46 %. There was no cytotoxic effect of quercetin at a concentration  $10^{-5}$  mol.l<sup>-1</sup> (Q-5). \*\*\* $p < 0.001$  for HY-5+Q-5 versus HY-5.

In our experiments, a significant protective effect of quercetin against the hypericin-induced cytotoxic effect was found. If we suppose that oxygen species play a role in the cytotoxicity of hypericin, our results are consistent with previous reports (Dewilde *et al.* 1996, Park *et al.* 1998), indicating that the generation of reactive oxygen species plays an important role in the light-induced phototoxicity of hypericin.

The role of oxygen in the light-induced cytotoxicity of hypericin and hypocrellin was clearly demonstrated by Park *et al.* (1998) who observed a significant reduction of the light-induced antiviral activity of hypericin and hypocrellin under hypoxic conditions. On the other hand, these authors found that the antiviral activity of hypocrellin was not observed at low oxygen levels at which hypericin retained measurable virucidal activity.

In conclusion, the results presented in this study have demonstrated that reactive oxygen species probably play an important role in the cytotoxic effect of hypericin. Similarly to other laboratories, we suggest that the mechanisms involving oxygen cannot explain all the aspects of hypericin activity, and that additional pathways, such as generation of free protons from excited states of hypericin can play an important role in the biological activity of hypericin.

### Acknowledgements

This work is partially supported by the Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences No 1/6034/99.

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