HYDROQUINONE INHIBITS GROWTH AND INDUCES APOPTOSIS OF U-251 MG CELLS

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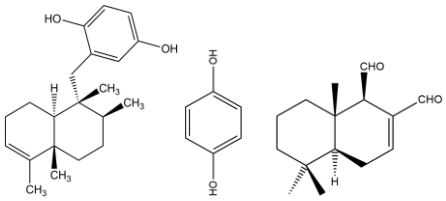
1. Introduction

Profound and selective cytotoxicity, penetration through the blood brain barrier along with lack of neurotoxic effects coupled with no side effects in humans at a dose of 3 mg/kg administered actually make the avarol chemical scaffold (Figure 1) an ideal candidate in design of new CNS drugs [1]. This study aimed to screen *in vitro* structure-activity relationship (along with selectivity) using avarol (followed by its structural elements) and the human malignant glioma U-251 MG cells.



A B C

Figure 1. - A. Avarol B. Hydroquinone C. Polygodial

2. Materials and methods

Avarol, a sesquiterpenoid hydroquinone isolated from the marine sponge *Dysidea avara*, was kindly provided by the colleagues from institute CNR-ICB in Pozzuoli-Naples (Italy), while hydroquinone and polygodial (Figure 1), along with the cells used, were purchased from commercial producers. Cytotoxicity and cell cycle analysis were determined using a colorimetric MTT assay and flow cytometry, respectively. Furthermore, mutagenicity was evaluated by comet assay, while the presence of hydroxyl radicals (•OH) was detected by EPR/ESR spectroscopy.

3. Results and discussion

Unlike doxorubicin, avarol and hydroquinone exhibited both significant cytotoxicity and selectivity (Table 1). However, none of these compounds were found to be cytotoxic towards normal (fetal, human) astrocytes.

Table 1. Cytotoxicity of avarol (AVL), hydroquinone (HQ), polygodial (PG), temozolomide (TMZ) and doxorubicin (DOX) towards selected cell lines

IC_{50} [μM]	AVL / HQ	PG	TMZ	DOX
U-251 MG, 96 h	1.22 ± 0.07 / 0.94 ± 0.01	>100	>100	1.01 ± 0.05
U-87 MG, 96 h	2.43 ± 0.12 / 1.87 ± 0.02	>100	>100	1.08 ± 0.03
normal astrocytes, 96 h	>100 / >100	>100	>100	0.23 ± 0.03

Polygodial, tested due to its chemical similarity with the avarol terpenoid moiety, did not show a cytotoxic effect of relevance towards U-251 MG cells (Table 1). Such a result has confirmed the importance of the hydroquinone part of the avarol scaffold for the screened cytotoxic activities.

In addition to this, proapoptotic effects were observed for avarol and hydroquinone, naturally occuring compounds (sub G1 peak, 96 h, IC_{50}, U-251 MG). More precisely, the percentage of specific apoptosis for hydroquinone was 5.38 fold higher, compared with avarol (Figure 2). On the other hand, the percentage of specific necrosis for hydroquinone (0.14 %) was almost 110 fold lower, compared with doxorubicin (15.38 %). No mutagenic effect was observed for both compounds. Finally, the identification of •OH has revealed one of the mechanisms of U-251 MG cytotoxic activity – production of Reactive Oxygen Species (ROS).

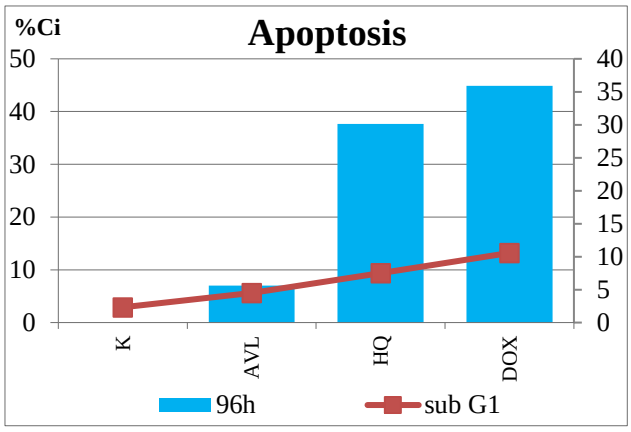


Figure 2. - Proapoptotic effects of avarol (AVL), hydroquinone (HQ) and doxorubicin (DOX) towards U-251 MG cells

4. Conclusion

This study has demonstrated a profound potential of hydroquinone in the design of new and more effective chemotherapeutic agent(s) specifically targeting malignant gliomas. Last but not least, the use of hydroquinone instead of avarol would undoubtedly contribute to the protection of *D. avara*, the sponge species which has been target of the marine natural product chemists for more than 40 years.

5. References

[1] S. De Rosa, G. Tommonaro, In "Studies in Natural Products Chemistry (Bioactive Natural Products)", Elsevier, 2012, Vol. 36, Chap. 6, pp. 163-211.