

MONITORING INFLUENCE OF SURGICAL STRESS ON FORMATION OF HYDROXYL RADICALS IN TUMOR BEARING RATS BY MEASURING SALICYLIC ACID METABOLITES

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In this article

- Hydroxyl radical (HO•) is one of the most harmful reactive oxygen species (ROS), which are formed in excess during oxidative stress.
- For measurement of HO• indirect methods are used determining aromatic acids hydroxylation products.

Keywords: oxidative stress, hydroxyl radical, salicylic acid, surgery, tumor, rat

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How to cite this article: MONITORING INFLUENCE OF SURGICAL STRESS ON FORMATION OF HYDROXYL RADICALS IN TUMOR BEARING RATS BY MEASURING SALICYLIC ACID METABOLITES. www.ifcc.org/ejifcc/vol12no2/borovic.htm

Introduction

Hydroxyl radical (HO•) is one of the most harmful reactive oxygen species (ROS), which are formed in excess during oxidative stress (shock, sepsis, trauma, surgery, hypoxia, ischemia-reperfusion, etc.) (1,2,3). For measurement of HO• indirect methods are used determining aromatic acids hydroxylation products. The most used is salicylic acid whose hydroxylation products 2,3-dihydroxybenzoic acid (2,3-DHBA) and 2,5-dihydroxybenzoic acid (2,5-DHBA) can be separated and quantified by HPLC method with electrochemical detection (4,5,6). As 2,5-DHBA can be produced also by enzymatic pathway, only 2,3-DHBA serves as a measure for HO• radical production (7).

The purpose of this work was to investi-

gate HO• formation in surgically treated tumor bearing rats and to see whether difference in HO• production can be seen between healthy and tumor bearing animals.

Materials and methods

Walker carcinoma tumor cells (107 live cells) were injected i.m. in the hind limb of male Wistar rats. Experiment was performed 6 days later when tumor was visible. Rats were divided in three groups: 1) anaesthetised only; 2) anaesthetised and operated (laparotomy); 3) anaesthetised and operated by laparotomy followed by ischemia-reperfusion (I/R) of tumor tissue caused by clamping ipsilateral iliac artery. The same treatment was done on healthy rats without tumor. Rats were treated per os with acetylsalicylic acid (ASA), 20 mg/kg body weight given through probe, anaesthetised with chloralhydrate (300 mg/kg) and operated (laparotomy). Plasma samples were collected after 60 minutes of ischemia and 30 minutes of reperfusion. For DHBA determination samples were extracted with hydrochloric acid and diethylether with 3,4-DHBA as internal standard. Separation was performed on 150 x 4,6 Waters Spherisorb ODS2 3 µm column using 7,48 mM sodium citrate/acetic acid mobile phase pH 4,6 with 3 % of methanol. Detection was done with electrochemical detector (ESA Detector Coulochem 2, with 5040 analytical cell model) set on 400 mV and flow on 0,6 ml/min. Chromatogram showing separation of DHBA was presented in **Figure 1**. Samples for SA determination were extracted with ethanol with 2,6-DHBA as internal standard.

Separation was done on the same column with UV detector set on 296 nm and flow of 1 ml/min. Mobile phase consisted of 7,48 mM sodium citrate/acetic acid pH 5,4 with 15 % of methanol. All analyses were done with Merck-Hitachi L-7100 HPLC system. Chromatogram showing separation of salicylic acid was presented in Figure 2.

Results were calculated as a percentage of salicylic acid from plasma and compared according to the Mann-Whitney test. Values with $p < 0,05$ were considered as significant.

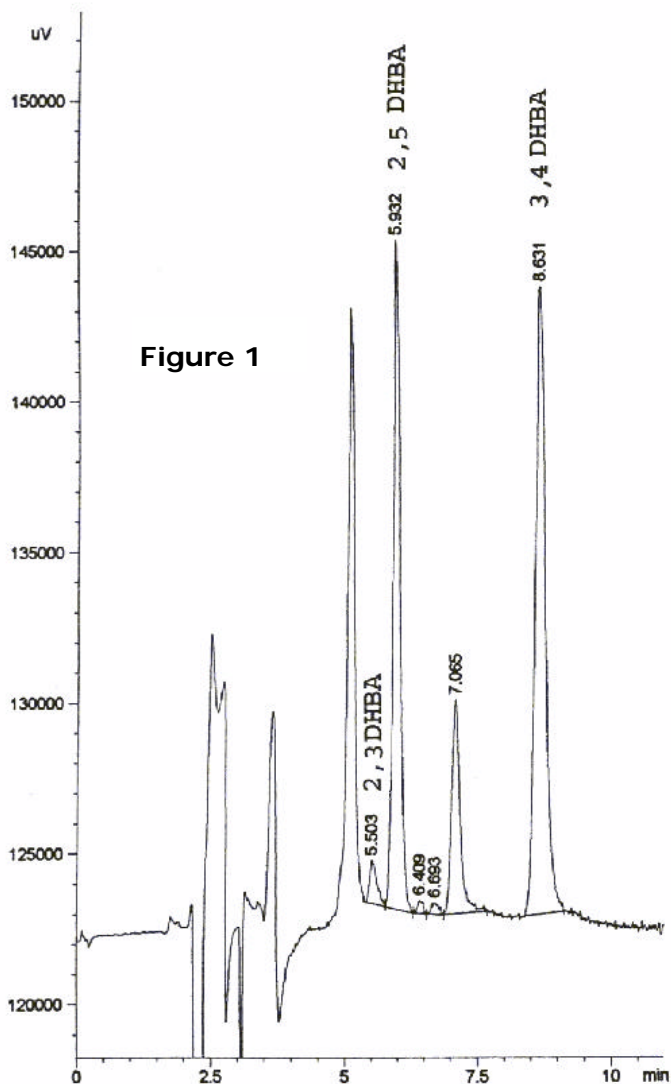
Results

The results obtained are summarised on Figure 3. Only minimal amount, i.e. approximately 0,01 - 0,05 % of SA was hydroxylated to 2,3-DHBA while 0,7 - 1,4 % was metabolised to 2,5-DHBA. In normal, healthy rats increase in 2,3-DHBA production was measured when they were surgically treated by laparotomy only ($p < 0,05$) or by laparotomy followed by I/R ($p < 0,05$). Such response was not noticed in tumor bearing rats where increase in 2,3-DHBA production was not observed neither in animals exposed to laparotomy only nor if laparotomy was followed by the tumor I/R. The difference observed between operated controls and tumor bearing rats was significant ($p < 0,05$). For 2,5-DHBA production, there was no difference observed between operated and non-operated rats for both control and tumor bearing animals.

Discussion

It is supposed that tumor cells are under persistent oxidative stress which seems to be beneficial to them, increasing metastatic potential and genetic instability, thus helping tumor cells to survive and progress (8,9,10). It is often assumed that mild oxidative stress caused by surgery can intensify metastases formation (11,12), although severe oxidative stress is not beneficial to the tumor cells and may even cause their destruction. Thus, additional ROS production in the I/R injury might be cytotoxic and cause cellular destruction (13,14). With our work we have not been able to see the increase in HO production in tumor bearing animals caused by laparotomy, which was seen in healthy surgically treated rats. Thus, it appears that systemic stress response caused by laparotomy was different in healthy and tumor bearing animals.

Difference in scavenger's levels and composition, and ROS production between normal and tumor bearing



rats cannot be excluded, while final appearances of these interactions can resemble perhaps even steady state as in unstressed animals. Since initial values of HO measured by 2,3-DHBA production were the same, we suppose that compensatory mechanisms (15), such as increased scavenger activity in tumor bearing organisms can be the cause of such response. Finally, due to the difficulties with per os application of ASA and consequently relatively ununiform 2,3-DHBA values present in the same group of animals, further evaluation of this model and a new approach with intravenous application seems reasonable. These experiments are already

in progress.

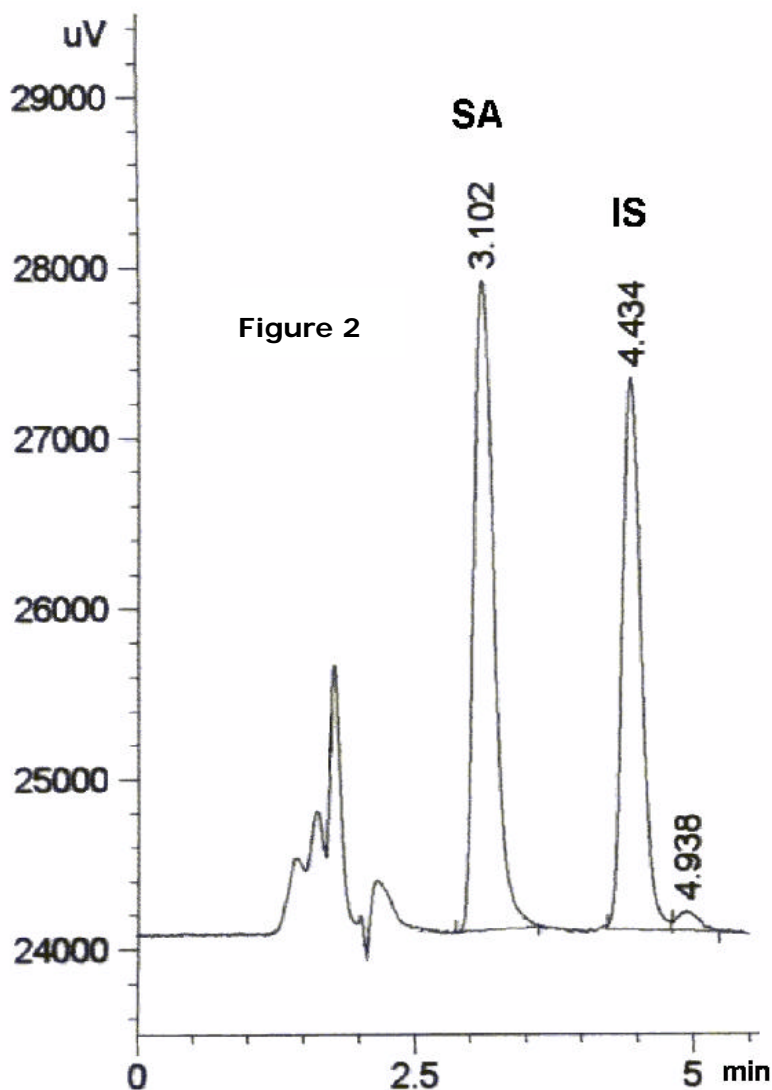
Acknowledgement

The authors would like to thank IFCC for providing Professional Scientific Exchange Scholarship to Ms. Suzana Borovic that made this work possible. Many sincere thanks to Univ. Prof. Dr. Gerhard Lanzer for generous hospitality in Department of Laboratory Medicine, Landeskrankenhaus, Graz, in which was done analytical part of this work. The support of the Croatian Ministry of Science and Technology is kindly acknowledged.

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