PHOTOACOUSTIC MEASUREMENT OF ETHYLENE AS A "REAL TIME" BIOMARKER OF LIPID PEROXIDATION PROCESS IN MICE

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RESUMEN

En algunas enfermedades como la porfiria intermitente aguda la acumulación de ácido 8-aminolevulínico (ALA) endógena con pH de 7 a 8 conlleva un proceso de enolización que produce radicales libres (O_2, OH, H_2O_2 y ALA). Se espera que la mayor parte de la ALA suministrada de manera exogena a un organismo sano sea convertida en protoporfirina IX (Pp IX), y finalmente al grupo hem, pero una pequeña acumulación de ALA iniciara un proceso de peroxidación lipídico debido a la generación de especies reactivas de oxígeno. Algunos de los productos finales comúnmente hallados en la peroxidación lipídica incluyen etano y pentano así como otros hidrocarbonos tal como etileno, propano, iso' y n' butano. Como estos gases son normalmente producidos a niveles de traza, se necesitan técnicas muy sensibles para detectarlos. En este trabajo se emplea el efecto peroxidativo del suministro de ALA de modo exógeno a ratones hembras CD1 estudiado por detección fotoacústica de trazas de gas. El etileno exalado por los ratones inoculados con ALA alcanza niveles de pocos ppb V 30 minutos después de recibir ALA.

ABSTRACT

In some diseases like the acute intermittent porfiria and lead poisoning the accumulated endogenous δ-aminolevulinic acid (ALA), with a 7 to 8 pH, undergoes an enolization process that produces free radicals (O_2^-, OH, H_2O_2 and ALA^-). It is expected that most of exogenously-supplied ALA in a healthy organism will be converted into protoporphyrin IX (PpIX), and ultimately to the hem group, but a small accumulation of ALA, when present, will initiate a lipid peroxidation process due to generation of oxygen reactive species. Some of the end products commonly found in lipid peroxidation include ethane and pentane, as well as other hydrocarbons like ethylene, propane, iso- and n-butane, isopentane and isobutene. As these gases are normally produced at trace levels, sensitive techniques are needed for their measurement. In this work, the peroxidative effect of exogenously-supplied ALA in female CD1 mice has been studied by photoacoustic trace gas detection. Exhaled ethylene from ALA-inoculated mice reached levels of the order of few ppbV for approximately 30 minutes, after supplying of ALA.

INTRODUCTION

Lipid peroxidation and free radical reactions in human disease and toxicology have increasingly attracted the attention of a number of authors. Probably the evidence most frequently quoted in support of the involvement of free radical reactions in tissue damage by disease and toxins is the measurement of elevated end-products [1]. In addition to ethane and pentane, other hydrocarbons like ethylene, propane, iso- and n-butane, isopentane and isobutene have been measured in studies of the peroxidative effect of a number of chemicals. [2]. The previous reported experiments on measuring the ethylene concentration as a biomarker of lipid peroxidation process were performed in a closed volume, so that these experiments could only give an idea of the amount of exhaled ethylene after long periods of integration time. The laser-driven photoacoustic detection method has proven to be very well suited for the on-line detection of trace gases emitted from biological material. [3]. Exhaled ethylene, used as a biomarker for lipid peroxidation in the skin of human subjects exposed to ultraviolet radiation, has been measured by this technique [4].

EXPERIMENTAL METHODS

The used system is a photoacoustic spectrometer with a CO_2 waveguide laser which emits radiation at wavelengths between 9 and 11 μm . With this laser it is possible to obtain approximately 80 lines distributed in four branches. The photoacoustic cell operates in an intracavity mode, so we can achieve

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more laser power through the studied gas. Similar systems have been reported before. [6].

For the study of the exhalations of the mice, it was developed a system to supply, clean, warm, split and filter the carrying air with the gas traces from the animals. A diaphragm pump supplies the air, the pumped air goes through a catalizer, so we have a "zero air supply". Before the air enters the cuvett, it passes through a gas heater, which allows to keep an appropriate temperature of the mice environment. The cuvett was built of pyrex glass, it has two holes permitting the entrance of clean air and the exit of the air with the exhalations, besides there is an additional entrance to inject from outside the gas system the studied mouse.

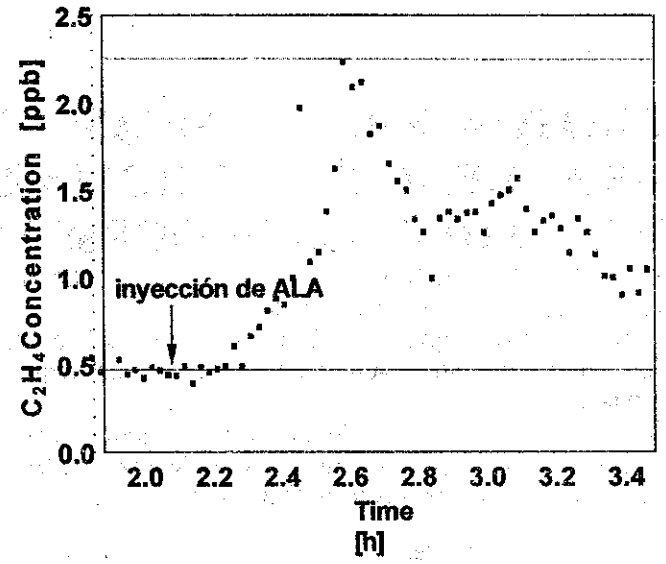
After the air passes the mouse cuvett, it is divided, because for the photoacoustic analysis only a small part of the flow is needed. The gas flow of few l/h to be measured goes through a several scrubbers and a cooling trap in order to absorb CO_2, NH_3 and H_2O molecules which interfere with the ethylene measurements.

Female CD-1 mice 4-5 years old were used. The mice were anesthetized with pentobarbital (90 mg/Kg) before introducing them inside the cuvett. After reaching the background level of ethylene emission the mice were intraperitoneally injected with 0, 80, 160, or 320 mg/Kg of ALA without opening the cuvett.

RESULTS

The ethylene concentration in the exhaled air starts to increase after some time depending on the dose of ALA administered to the mouse. Also, the total amount of ethylene emission induced by lipid peroxidation processes depends on the ALA dose. Graphic 1 presents a typical curve of exhaled ethylene concentration as a function of time. The arrow indicates the moment that the ALA was injected.

The results obtained by the photoacoustic method were compared with those obtained by the reactive substances to tiobarbituric acid technique. This comparison shows a very good agreement between both results.



Graphic 1. Ethylene emission after ALA administration.

CONCLUSIONS

In the present work, the authors suggest and have proven the feasibility to monitor the ethylene emission of laboratory animals using a photoacoustic spectrometer as a real time biomarker of the lipid peroxidation process.

This proposed method could be applicable as a new powerful technique in preclinical trials of new pharmaceutical drugs, which if administered in excess could lead to a delivery of reactive species detectable by ethylene emission monitoring.

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