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Antioxidant capacity of 5-Fluorouracile and new fluorinated uracil derivates

Capacidad antioxidante del 5-fluorouracilo y nuevos derivados uracilofluorados

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ABSTRACT

Oxidative stress is associated with multiple pathologies such as cancer and can exacerbate the development of them. In this work, we have studied the antioxidant capacity of 5-Fluorouracile (5-FU) which is an antineoplastic drug that is used in the treatment of colorectal cancer. 5-FU is a compound that has a chemical structure similar to uracil and is also fluorinated. New fluorinated derivates previously obtained in our laboratory were tested to study its antioxidant activity. All the compounds analyzed were able to inhibit lipid peroxidation when used in concentrations of 10 μM.

KEYWORDS: *antioxidants, MDA, lipid peroxidation, antineoplastic, cancer.*

RESUMEN

El estrés oxidativo está asociado a múltiples patologías, como el cáncer, y puede agravar el desarrollo de estas. En este trabajo hemos estudiado la capacidad antioxidante del 5-fluorouracilo (5-FU), que es un fármaco antineoplásico que se utiliza en el tratamiento del cáncer colorrectal. El 5-FU es un compuesto que tiene una estructura química similar al uracilo y además está fluorado. Nuevos derivados uracilofluorados que se habían obtenido previamente en nuestro laboratorio fueron testados para estudiar su actividad antioxidante. Todos los compuestos analizados fueron capaces de inhibir la peroxidación lipídica cuando se utilizaron a concentraciones de 10 μM.

PALABRAS CLAVE: *antioxidantes, MDA, peroxidación lipídica, antineoplásico, cáncer.*

INTRODUCTION

Oxidative stress is defined as the alteration produced by the imbalance between oxidants and antioxidants species (Sies, 1986). It can be caused by an excess of oxidants, antioxidant deficiency, or both factors simultaneously. Currently, there is a growing interest in oxidative stress because it can lead to cell death and contribute to the development of multiple pathologies (Serero et al., 2008). Some of these diseases are cancer, type 2 diabetes mellitus, atherosclerosis, myocardial infarction, acute pancreatitis, Parkinson's and Alzheimer's disease among others (Wang et al., 2014; de Sa Junior et al., 2017; Miletic et al., 2017).

Colorectal cancer is the fourth leading cause of cancer death in the world. It presents an incidence of 1.4 million of cases per year with a death rate of 700,000 people (Bhardwai et al., 2017). In colorectal cancer, a widely antineoplastic drug used is the fluorinated uracil molecule 5-Fluorouracile (5-FU) (Yoshitani and Takashima, 2009). This molecule is an antineoplastic antimetabolite of the pyrimidine base uridine. It acts as a false substrate in the synthesis process of the essential constituents of nucleic acids, causing the synthesis of an anomalous DNA. It is indicated in a large variety of tumors. It is used orally or intravenously. It is absorbed in the intestine and is rapidly distributed throughout the body. It is metabolized in the liver giving rise to its active metabolite being eliminated by several mechanisms (Woloch et al., 2012).

Since oxidative stress and lipid peroxidation have been causally implicated in the pathogenesis of cancer and the previous cited pathologies, the determination of remaining products of lipid peroxidation, such as MDA, has been accepted and widely used as a marker of this process (Bosch-Morell et al., 1998; Bunnag, 2006; Surekha et al., 2007). For this reason, the aim of our study is to assess the antioxidant capacity observed by the inhibition of lipid peroxidation by 5-FU and other new fluorinated uracil derivates previously synthetized in our laboratory: 6-[Difluoro(phenyl)methyl]-3-(2,4,6-trifluorophenyl)pyrimidine-2,4(1H,3H)-dione (S-81), 3-[2-Chloroethyl]-6-[difluoro(phenyl)methyl] pyrimidine-2,4(1H,3H)-dione (S-86), 6-[Difluoro(phenyl)methyl]-3-(4-trifluoromethoxyphenyl)pyrimidine-2,4(1H,3H)-dione (S-123). Padhye and collaborators (2010) observed that the fluorinated chalcones were more potent as antioxidants and as anti-proliferative drugs than the initial compounds with phenolic hydroxyl groups, their hydroxyl counterparts which were substituted by fluor. (Padhye et al., 2010).

METHODS

Animals

Male C57BL/6 mice (20-25 g) were used. Animals received human care in accordance with guidelines established in Spanish legislation (Royal Decree RD 53/2013). The protocol was approved by the Experimental Animal Ethics Committee CEU Cardenal Herrera University.

Lipid peroxidation inhibition

Lipid peroxidation inhibition was determined by measuring MDA concentration in samples of C57BL/6 mouse liver homogenates with an HPLC system (Waters) of the CEU Cardenal Herrera University following Richard's method (Romero et al., 1998). 5 mL of liver homogenate were adjusted to a final protein concentration of 12 mg/mL. A range of concentrations between 0 and 10 μM of fluorinated uracil derivates and peroxidation inducers were added to the homogenates and incubated at 37 ºC for 2 h. After incubation homogenates were stored at –80 ºC until MDA analysis.

The chromatographic separation column used was a Kromasil C18 5μm of 250 x 4.6 mm (Análisis Vínicos S.L.). The flow of the mobile phase was 1 mL/min. The excitation wavelength used was 532 nm and the emission wavelength was 553 nm. The fluorescence detector voltage used was 600 and the response was 2 seconds. The mobile phase was prepared with 50 mM of phosphate buffer at pH 6.0 and methanol (580 mL of buffer and 420 mL of methanol). Then, it was filtered through a membrane filter (Scheicher und Schuell) of 0.45 μm pore and 47 mm diameter. The calibration stock solution was prepared daily and consists of a 20 mM concentration of 1,1,3,3-tetraethoxypropane in absolute ethanol. The working solution consists of a preparation of thiobarbituric acid (TBA) (0.37 %) and perchloric acid (6.4 %), 2: 1 v/v respectively. The working solution was also prepared daily. 0.1 mL of the sample and 0.75 mL of the working solution are mixed well and kept for 60 minutes in a 95 °C water bath. After this time the mixed was cooled to 4 °C for 10 min in order to stop the reaction, and centrifuged for 10 min at 12,000 rpm. Until its injection into the HPLC unit, the tubes were kept at a constant temperature of 4 ºC. Since the MDA-TBA adduct is unstable at neutral pH, each sample was neutralized with 0.7 M of potassium hydroxide a few minutes before injection into the HPLC equipment. Immediately after neutralizing, centrifuge for 1 min to help precipitate insoluble salts that could interfere in the determination and proceed, after filtering with non-sterile syringe filters (3 mm, 20 microns, Teflon membrane, supplied by Corning Laboratory Sciences Company), to inject into the HPLC equipment. In each experiment, a blank and a standard calibration curve (0, 0.25, 0.5, 1 and 2 μM) were prepared. The area of the peak obtained is directly proportional to the concentration of MDA in the sample, which was calculated by intrapolation in the regression line obtained with the standards.

Total antioxidant capacity

Total antioxidant capacity (TAC) of 5-FU was measured by the ELISA kit from Cayman-chemical™ which is based on the ability of antioxidants to inhibit the oxidation of ABTS (2,2`- Azino-di-[3-ethylbenzthiazoline sulphonate]). Antioxidant capacity was expressed in TEAC (Trolox Equivalent Antioxidant Capacity) units.

Statistical analysis

Results are shown as mean ± SD. Statistically significant differences in mean values were tested by ANOVA test using SPSS Software. Differences were considered significant when $p < 0.05$.

RESULTS

We have observed a significant decrease in MDA production of 58.12 % in S-86, 44.61 % in S-123, 24.11 % in 5-FU and 10.83 % in S-81 in the sample with highest concentration (10 µM) (Figures 1-2). 5-FU also showed a TAC of 0.68 TEAC.

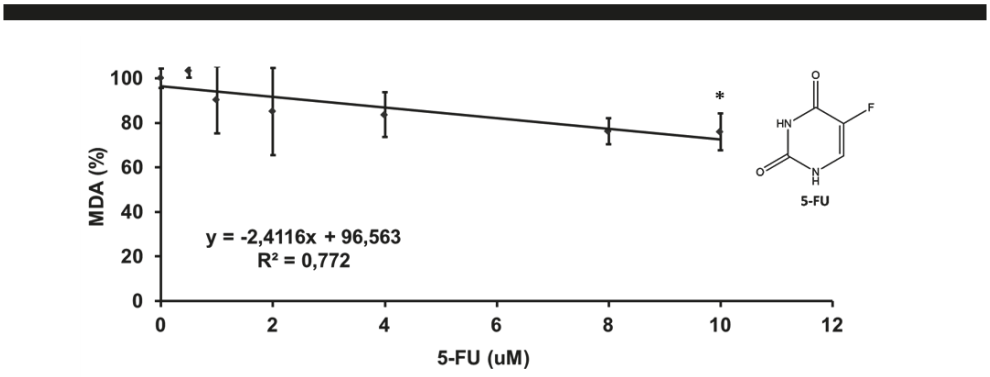

Figure 1. MDA production in presence of 5-FU in samples of C57BL/6 mouse liver. Concentrations used of 5-FU were 0, 2, 4, 6, 8, 10 µM. Statistical significance is indicated as *$p < 0.05$ vs 0 µM.

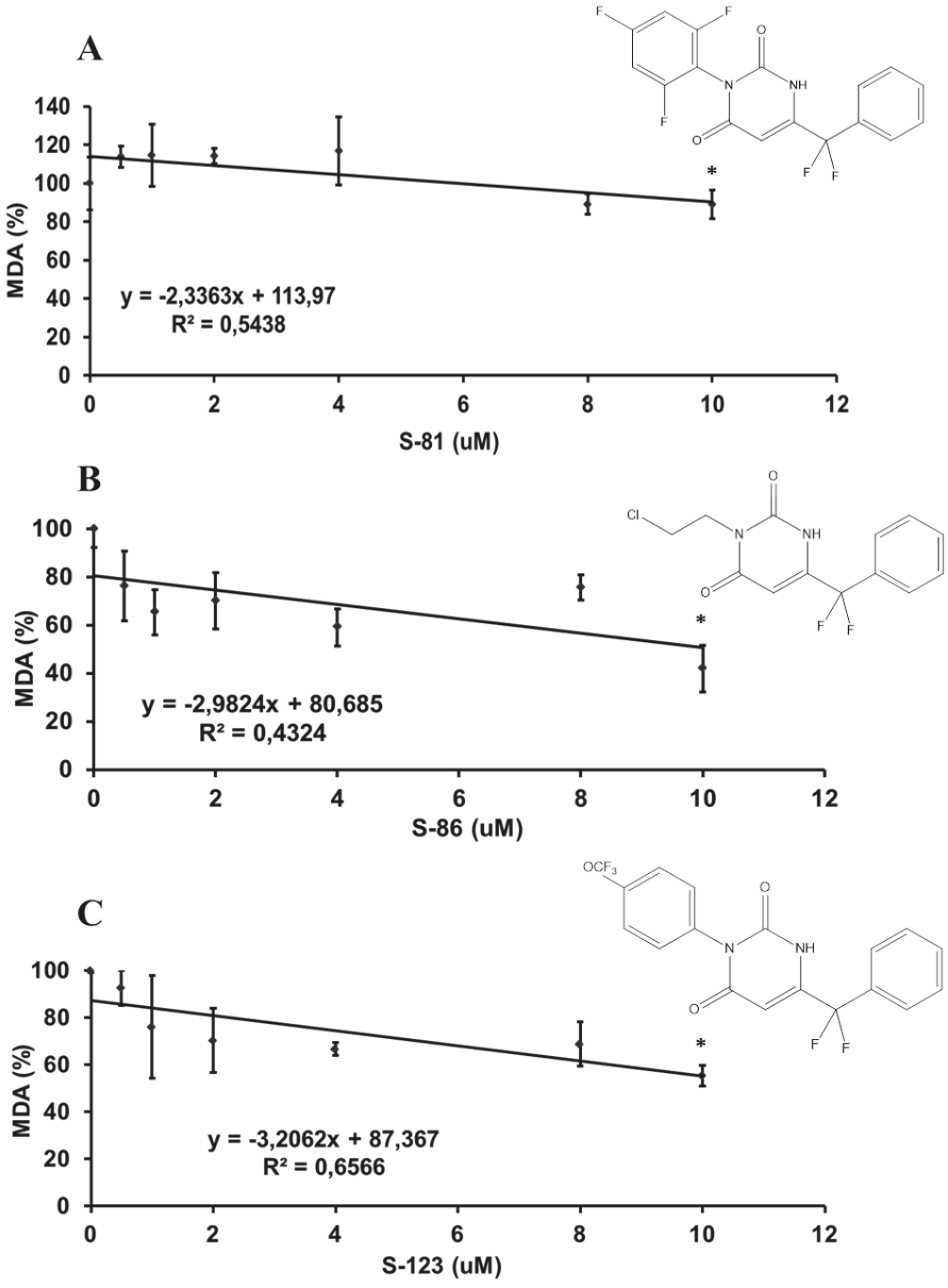

Figure 2. MDA production in presence of S-81 (A), S-86 (B) and S-123 (C) in samples of C57BL/6 mouse liver. Concentration of the three molecules studied were 0, 2, 4, 6, 8, 10 µM. Statistical significance is indicated as *p < 0.05 vs 0 µM.

DISCUSSION

Several studies have focused their researches on the antioxidant activity of various uracil derivatives (Akhatova et al., 2011; Spanou et al., 2011). In our studies, we provide new uracil derivates (S-81, S-86 and S-123) that decrease the percentage of MDA concentration indicating an antioxidant capacity. Since oxidative stress is associated with cancer, 5-FU could be an interesting drug in antitumor therapy not only for its antineoplastic properties, but also for its antioxidants too. In this way, 5-FU could provide the advantage of the combination in the same drug preparation of two beneficial effects for the treatment of cancer. In this line, it would avoid pharmacokinetic problems and interactions due to the separate administration and combined of an antioxidant drug and another antineoplastic drug. This also implies an increase in the comfort level of the patient who receives the treatment.

CONCLUSION

It was decided from the very beginning of our research work to study the fluorinated compounds, instead of the phenolic ones without fluor and with only hydroxyl groups. As it can be found in other studies the fluorinated compounds are more potent antioxidant and anti-proliferative drugs than their hydroxyl counterparts. 5-FU and the new fluorinated uracil derivates (S-81, S-86 and S-123) studied have a significant antioxidant capacity. Specifically, the antineoplastic 5-FU has both biological and antioxidant activity *per se.*

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