Toxicology Letters

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A mechanistic study on the cardiotoxicity of 5-fluorouracil in vitro and clinical and occupational perspectives

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HIGHLIGHTS

- In the present study, we study what doses can determines direct cardiotoxic effects of 5-fluorouracil on rat cardiocytes.
- We have found that 5-FU induced 50% growth inhibition at 72 h with concentrations of 400 μM on H9c2 cardiocytes.
- We have found that the addition of Levofolinic Acid (LF) to 5-FU potentiated the growth inhibition induced by 5-FU.
- The growth inhibition induced by 5-FU alone or in combination with LF in cardiocytes was paralleled by an increase of Tbars and end products of NO.
- Our data suggest that 5-FU can also induce cardiocyte damage by oxidative stress.

ARTICLE INFO

ABSTRACT

Fluoropyrimidines are key agents for the treatment of gastrointestinal tract adenocarcinomas. The possible cardiotoxic effects in patients and occupationally exposed workers are multifactorial and remain a puzzle to solve for investigators. In the present study, we study what cell death pathways and what doses can determine direct cardiotoxic effects of 5-fluorouracil (5-FU) and doxorubicin (DOXO) on rat cardiocytes (H9c2) and a human colon adenocarcinoma (HT-29) cell line, already reported to be sensitive to 5-FU. We have found that 5-FU induced 50% growth inhibition (IC:50) at 72 h with concentrations of 400 μM and 4 μM on H9c2 and HT-29, respectively. Moreover, we have found that the addition of Levofolinic Acid (LF) to 5-FU potentiated the growth inhibition induced by 5-FU. The growth inhibition induced by 5-FU alone or in combination with LF in cardiocytes was paralleled by an increase of thiobarbituric acid-reactive species (Tbars) and end products of nitric oxide (NO) suggesting the increase of the oxidative stress status in cardiocytes. Interestingly, these effects were strongly potentiated by the addition of LF, a biochemical modulator of 5-FU activity.

Our data suggest that agents such as 5-FU different from anthracyclines, conventionally related to the induction of cardiotoxic effects, can also induce cardiocyte damage paralleled by oxidative stress. The strategies based upon the use of scavengers could be used in order to prevent this effect.

1. Introduction

More than two decades after its synthesis (Duchinsky et al., 1957), 5-fluorouracil (5-FU) remains a key agent to several chemotherapy regimens, particularly for the treatment of gastrointestinal tract adenocarcinomas in combination with levofolene (LF) (Ashraf et al., 2013; Rödel et al., 2012). On these bases, there is no reason in studying 5-FU effect without combining it with LF.

Unfortunately, like most chemotherapeutic agents, 5-FU alone or, more markedly, in combination with LF has also numerous toxic effects as diarrhea, mucositis, myelosuppression, and thrombophlebitis of peripheral veins (Sorrentino et al., 2012). The wide range of cardiotoxicity related to 5-FU occurs less frequently but are typically more serious. The most severe cardiac complications are heart failure (Brestescher et al., 1995), arhythmia (Weidmann et al., 1995; Eskilsson et al., 1988; Aziz et al., 1998) and myocardial ischemia (Gorgulu et al., 2002).

This risk is also possible in the healthcare workers during the phases of manipulation of antiblastic drugs. In fact, several scientific studies have shown that workers can be exposed to cardiotoxic

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drugs such as doxorubicin, epirubicin, cyclophosphamide and 5-FU (Connor et al., 2010; Lamberti et al., 2014).

The understanding of the molecular mechanisms at the basis of the cardiotoxic effects of 5-FU could be useful in order to determine possible pharmacological strategies in order to prevent this deleterious side effect. Moreover, studies on the toxic effects of 5-FU alone or in combination with LF on in vitro models of cardiocytes are presently limited. In this view, the setting of an experimental model in which the molecular mechanisms of toxicity can be easily studied could be useful in order to detect new targets and strategies to prevent cardiac side effects of anti-cancer agents without reducing their anti-cancer activity. The availability of a cheap cardiac in vitro model for the study of the molecular pathways disrupted by the treatment with 5-FU could be a useful tool to find new targets to be validated in a preclinical in vivo model. In fact, it is not still clear how to correctly study in preclinical and clinical model the occurrence of the molecular events of 5-FU-induced cardiotoxicity and therefore the cardiopreventive approaches are still a black hole.

The following different mechanisms of the cardiac toxic effect induced by 5-FU have been proposed: coronary artery spasm, autoimmune-mediated injury of the myocardium, endothelial damage, thrombogenic effects or thrombus formation, global dysfunction, accumulation of metabolites and direct myocardial toxicity causing necrosis (Sorrentino et al., 2012).

In the present manuscript, we have studied the concentrations of 5-FU and doxorubicin (DOXO) that can determine direct cardiotoxic effects in a model of rat cardiocytes (H9c2). Moreover, we have studied the oxidative stress changes and the mitochondrial membrane potential transition in order to find biochemical correlates to the cardiac damage.

2. Materials and methods

2.1. Materials

RPMI, DMEM, and FBS were purchased from Flow Laboratories (Milan, Italy). Tissue culture plasticware was from Microtech (Naples, Italy).

2.2. Cell culture and proliferation

The rat cardiocytes (H9c2) cell line and the human colon adenocarcinoma (HT-29) cell line obtained from the American Type Tissue Culture Collection, Rockville, MD, grow in DMEM and RPMI1640, respectively, supplemented with heat inactivated 10% FBS, 20 mM HEPES, 100 U/ml penicillin, 100 mg/ml streptomycin, 1% L-glutamine and 1% sodium pyruvate. Both cell lines were grown in a humidified atmosphere of 95% air, 5% CO_2 at 37 °C. Proliferation of H9c2 and HT-29 cell lines was performed in the presence of 5-FU (2–1250 μM), alone and in combination with 10^{-4} M of Levofolene (LF), and of DOXO (0.1–6.4 μM), by MTT assay as previously described (Chiosi et al., 2007).

2.3. Nitrite assay

NO is rapidly converted into the stable end products nitrite (NO_2^-) and nitrate. Nitrite was measured by the Griess reaction as previously reported (Titheradge, 1998). The nitrite assay used in this work was described in Gomez-Monterrey et al. (2013).

2.4. Thiobarbituric acid-reactive species (Tbars) levels

Samples were incubated with 0.5 ml of 20% acetic acid, pH 3.5 and 0.5 ml of 0.78% aqueous solution of thiobarbituric acid. After heating at 95 °C for 45 min, the samples were centrifuged at 4000 rpm for 5 min. In the supernatant fractions Tbars were quantified by spectrophotometry at 532 nm (Gomez-Monterrey et al., 2013). Results were expressed as Tbars μM/μg of serum protein. Each data point is the average of triplicate measurements, with each individual experiment performed in duplicate as previously reported in Caraglia et al., 2011.

2.5. Determination of mitochondrial potential through FACS analysis (MitoTracker Red)

After 24, 48 and 72 h from the beginning of the established treatments cells were detached, centrifuged at $1200 \times g$ for 5 min and resuspended in PBS. MitoTracker Red probe was added at a final concentration of 100 nM. The latter does not emit fluorescence if it is not internalized in living cells. Once in the cytoplasm of living cells the probe is oxidized in a series of reactions in presence of ROS and if mitochondrial pores are opened due to the mitochondrial potential transition, the probe enters into the mitochondria. The positively charged probe binds the mitochondrial proteins and emits a red fluorescence. After the incubation with the probe for 20 min at room temperature in the dark, cells were centrifuged, resuspended and analysis of red fluorescence was performed by flow cytometry (FACScan, Becton Dickinson). For each sample, 2×10^4 events were acquired. Analysis was carried out by triplicate determination on at least three separate experiments.

2.6. Statistical analysis

All data are expressed as mean ± SD. Statistical analysis was performed by analysis of variance (ANOVA) with Neumann–Keul's multiple comparison test or Kolmogorov–Smirnov where appropriate.

3. Results

3.1. Effects of DOXO and 5-FU on H9c2 and HT-29 on cell proliferation

We studied the effect of increasing concentrations of DOXO and 5-FU in presence or not of LF on growth inhibition of HT-29 and H9c2 cells by MTT assay as described in Section 2. We have found a dose and time-dependent (data not shown) growth inhibition in both cell lines. The IC:50 (50% inhibitory concentration) was reached after 72 h by pharmacological treatment in both cell lines. In details, the values of 5-FU were 4 μM and 400 μM in HT-29 and H9c2, respectively, after 72 h from the beginning of the treatment (Fig. 1). Moreover, LF potentiated growth inhibition induced by 5-FU. In fact, IC:50 of HT-29 and H9c2 cells was 2 μM and 43 μM, respectively, (Fig. 1).

These results suggest, as expected, that the colon cancer cell line HT-29 was more sensitive to 5-FU than H9c2 normal cells (Fig. 1). Interestingly, these concentrations of 5-FU can be reached in vivo after the routinely used ways of administration of this agent in the clinical practice (Büchel et al., 2013).

On the other hand, H9c2 cells appeared to be more sensitive to DOXO than HT-29. In fact, the IC:50 of DOXO was 0.12 μM and 0.31 μM on H9c2 and HT-29, respectively, (Fig. 1).

3.2. Effects of 5-FU ± LF on peroxidation of membrane lipids and NO_2^- formation

In order to evaluate the oxidative stress in cardiocytes and in human colon adenocarcinoma cells treated with the different anti-cancer agents, we have assessed the intracellular Tbars in order to add data about the membrane lipid peroxidation. Tbars levels were almost undetectable in untreated cardiocytes while the treatment of the cells with 400 μM of 5-FU for 72 h induced the formation
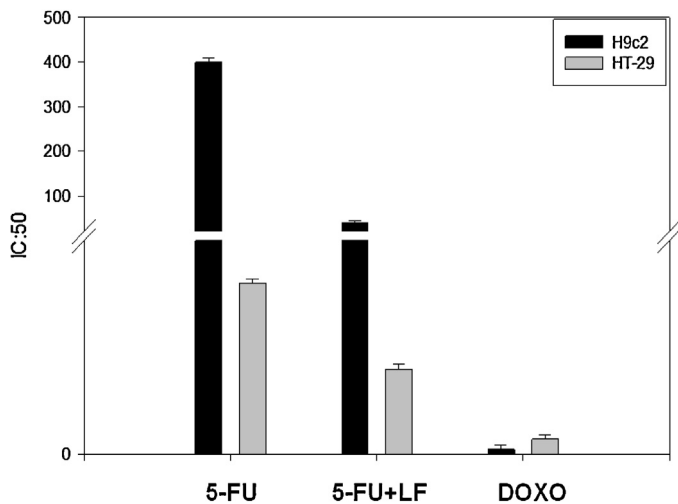
Fig. 1. *Antiproliferative effects of 5-FU and/or LF or DOXO on H9c2 and HT-29 cells.* The cells were seeded and treated with different concentrations of 5-FU or 5-FU + LF or DOXO for 72 h and thereafter the cell growth inhibition was evaluated with viability MTT assay as described in Section 2. Thereafter, the concentration of the different drugs inducing 50% growth inhibition (IC:50) after 72 h of treatment was calculated and expressed in columns. The experiments were performed three different times. The bars represent means ± SD of three independent experiments.

Fig. 3. *Effects of 5-FU and/or LF on Tbars in HT-29 cells.* The cells were seeded in six multi-well plates at the density of 15×10^4 cells/plate and, after attachment, they were incubated with 5-FU alone and in combination with LF for 72 h, as described in the text. Tbars levels in the different experimental conditions were determined as described in Section 2. The bars represent means ± SD of three independent experiments. Asterisks indicate significant difference between 5-FU-treated vs. 5-FU + LF-treated cells ([**]$P < 0.003$) and untreated vs. 5-FU-treated cells ([*] $P < 0.05$).

of about 1.1 μM/μg of proteins of Tbars (Fig. 2). Interestingly, the exposure of cardiocytes to 43 μM of 5-FU in combination with 10^{-4} M of LF caused the formation of about 6.6 μM/μg of proteins of Tbars thus potentiating the effects induced by 5-FU alone (Fig. 2). On the other hand, Tbars levels in human colon adenocarcinoma cells treated with 4 μM of 5-FU for 72 h were about 0.015 μM/μg of proteins of Tbars while the treatment of the cells with 2 μM of 5-FU in combination with 10^{-4} M of LF resulted in a smaller increase of Tbars levels with 0.017 μM/μg of proteins of Tbars (Fig. 3). In the same experimental conditions, we have also evaluated the formation of NO_2^- in the cell medium. We have found that NO_2^- levels
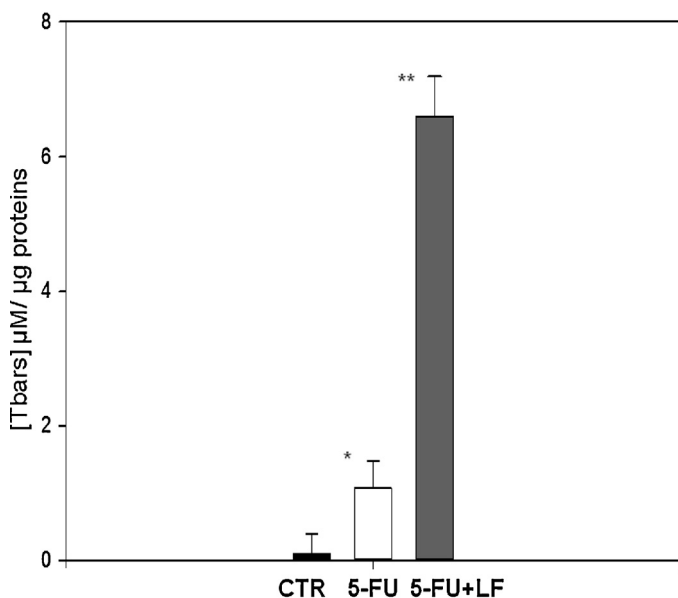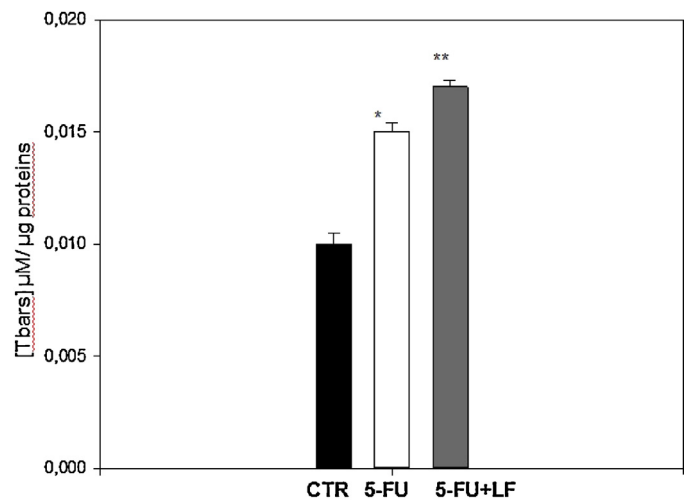
were about 0.9 nM/μg proteins in untreated cardiocytes while the treatment of the cells with 400 μM of 5-FU for 72 h induced the formation of about 2.0 nM/μg proteins of NO_2^- (Fig. 4). Again, the exposure of cardiocytes to 43 μM of 5-FU in combination with 10^{-4} M of LF potentiated the formation of NO_2^- that reached a value of about 8.3 nM/μg proteins suggesting a potentiation of the combination on the secretion of potentially detrimental agents for heart function such as NO_2^- (Fig. 4). On the other hand, NO_2^- levels in untreated colon adenocarcinoma cells were 0.017 nM/μg proteins while the treatment with 4 μM of 5-FU for 72 h induced a decrease in the formation of NO_2^- with 0.009 nM/μg proteins (Fig. 5). The treatment with 2 μM of 5-FU and 10^{-4} M of LF caused an additional decrease of NO_2^- levels reaching about 0.005 nM/μg proteins of

Fig. 2. *Effects of 5-FU and/or LF on Tbars in H9c2 cardiomyocytes.* The cells were seeded in six multi-well plates at the density of 25×10^4 cells/plate and, after attachment, they were incubated with 5-FU alone and in combination with LF, as described in the text, for 72 h. Tbars levels in the different experimental conditions were determined as described in Section 2. The bars represent means ± SD of three independent experiments. Asterisks indicate significant difference between 5-FU-treated vs. 5-FU + LF-treated cells ([**] $P < 0.003$) and untreated vs. 5-FU-treated cells ([*] $P < 0.05$).
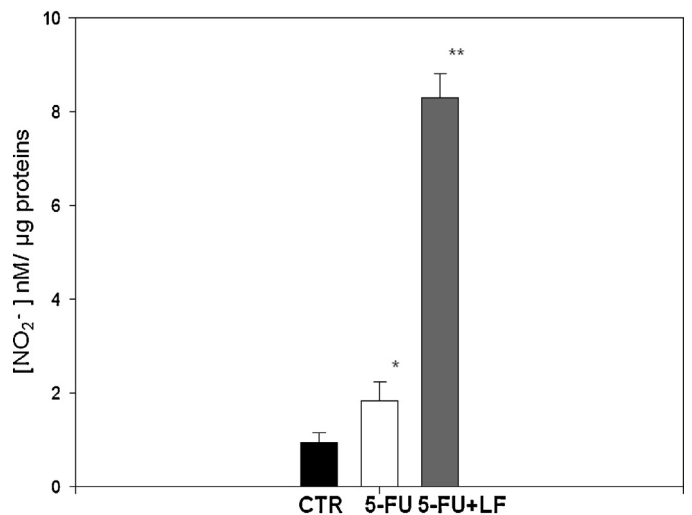

Fig. 4. *Effects of 5-FU and/or LF on NO_2^- secretion in H9c2 cardiomyocytes.* The cells were seeded in six multi-well plates at the density of 25×10^4 cells/plate and, after attachment, they were incubated with 5-FU alone and in combination with LF, as described in the text, for 72 h. NO_2^- levels in the different experimental conditions were determined in the cell medium as described in Section 2. The bars represent means ± SD of three independent experiments. Asterisks indicate significant difference between 5-FU-treated vs. 5-FU + LF-treated cells ([**] $P < 0.003$) and untreated vs. 5-FU-treated cells ([*] $P < 0.05$).

Fig. 5. *Effects of 5-FU and/or LF on NO_2^- secretion in HT-29 cells.* The cells were seeded in six multi-well plates at the density of 15×10^4 cells/plate and, after attachment, they were incubated with 5-FU alone and in combination with LF for 72 h, as described in the text. NO_2^- levels in the different experimental conditions were determined in the cell medium as described in Section 2. The bars represent means ± SD of three independent experiments. Asterisks indicate significant difference between 5-FU-treated vs. 5-FU + LF-treated cells ([**] $P < 0.003$) and untreated vs. 5-FU-treated cells ([*] $P < 0.05$).
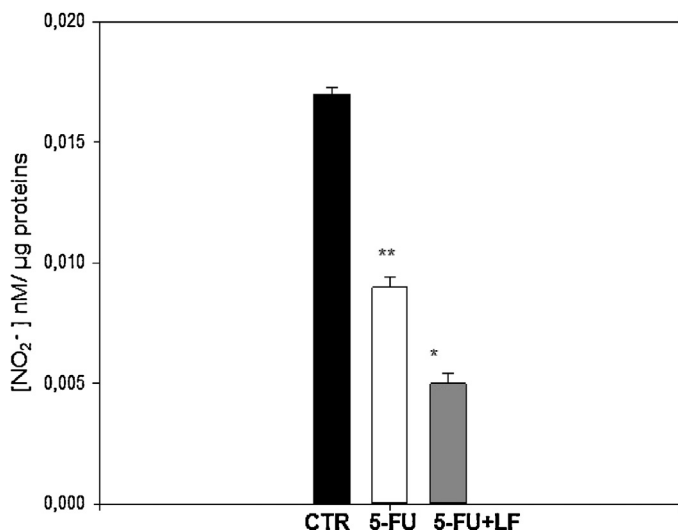
NO_2^- (Fig. 5). These data suggested that adenocarcinoma cells were less sensitive to damage induced by pharmacological treatments on peroxidation of membrane lipids compared to cardiocytes probably because of their tumor histogenesis.

3.3. Effects of 5-FU ± LF on mitochondrial membrane potential

Effects of either 5-FU, alone or in combination with LF, or of DOXO on membrane mitochondrial potential of H9c2 cells was evaluated through FACS analysis after MitoTracker labelling as described in Section 2. The fluorescence intensity was quantized at FACS and expressed as Mean Fluorescence Intensity (MFI) in percentage of positive control.

After 24 h form the beginning of the treatment 5-FU alone induced an about 40% increase of MFI and this effect was not changed by the addition of LF while DOXO caused an about 80% increase of MFI (Fig. 6). After 48 h, 5-FU alone induced an about 50% increase of MFI that was potentiated by the addition of LF (about 83% MFI increase). In the same experimental conditions, DOXO caused an about 120% increase of MFI (Fig. 6).

After 72 h from the beginning of the treatment, the fluorescence levels in cells exposed to 5-FU with or without the addition of LF resumed to those of untreated cells while DOXO-treated cells showed still an about 60% increase of MFI if compared to untreated cells. Interestingly, LF alone did not induce relevant changes in MFI in all the time points evaluated in our experimental conditions (Fig. 6).

4. Discussion

Incidence of cardiotoxicity in patients treated with 5-FU varies between 1.2 and 18% with a mortality rate between 2.2 and 13.3% (Burger and Mannino, 1987; Teixeira et al., 2004; Meyer et al., 1997). The appearance of toxic effects is also possible in exposed workers, because 5-FU has been reported as one of the more concentrated chemotherapeutic drug in the work areas (950 ng/cm^2) (Connor et al., 2010). Yoshida et al. (2011) showed that, in
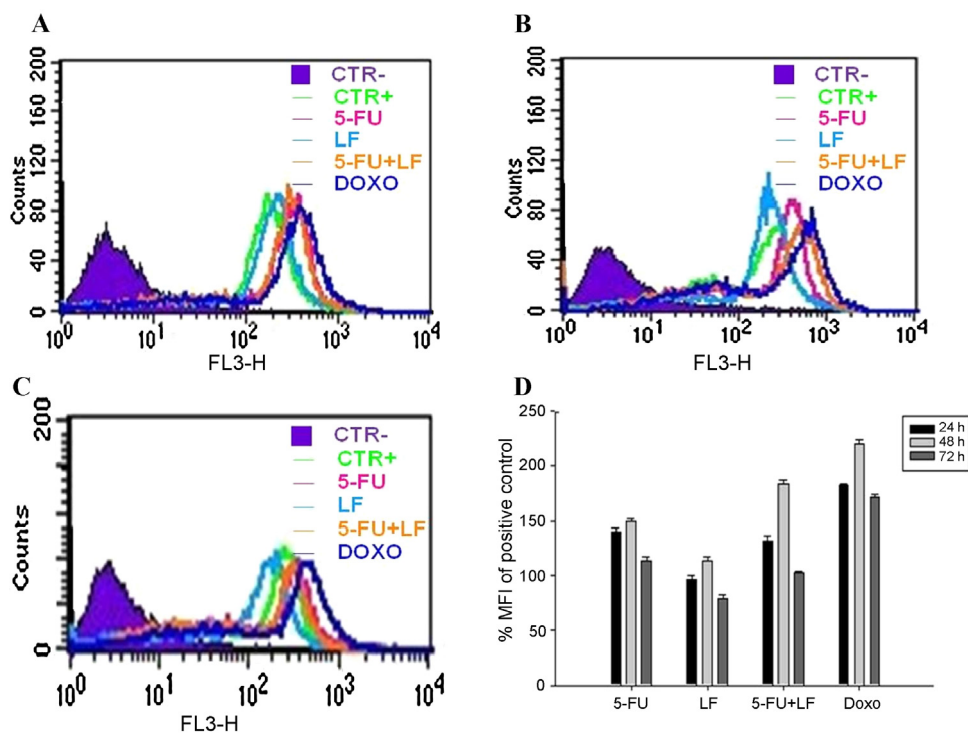

Fig. 6. *Analysis of the mitochondrial membrane potential in cells treated with 5-FU and/or LF or DOXO on H9c2 cardiocytes.* The cells were seeded and treated with concentrations of 5-FU or 5-FU + LF or DOXO corresponding to their respective IC:50s for 24 h (A), 48 h (B) or 72 h (C) and then the analysis of the mitochondrial membrane potential was performed after MitoTracker labelling as described in Section 2. CTR$^-$ indicates untreated cells not labelled with MitoTracker; CTR$^+$ indicates untreated cells labelled with MitoTracker. The MFI changes have been evaluated as percentage of CTR$^+$ and were represented as columns (D). The experiments were performed at least three times. The bars represent means ± SD of three independent experiments.

5 hospitals in Japan, 5-FU had the highest concentration detected in working table, on floor and in air-conditioner filter (respectively, 43 ± 44 ng/m^2; 5.2 ± 4.2 ng/m^2 and 4600 ng).

Extensive studies of biological monitoring, in exposed workers, for 5-FU have not been made and therefore it is not possible to assess accurately the level of exposure of the medical staff; however, other toxic effects more easily correlated with the exposure such as myelosuppression and hepatic toxicity have already been highlighted in a population of 5800 workers (Caciari et al., 2012).

Although mortality is also uniformly higher in subjects with underlying heart disease, including coronary artery disease (CAD), structural heart disease, and cardiomyopathies (Labianca et al., 1982; Rezkalla et al., 1989), the mechanism of cardiotoxicity of 5-FU remains poorly defined.

The main cause of identified cardiotoxicity is a direct effect on vascular endothelium that involves endothelial nitric oxide (NO) synthase and leads to coronary spasms and endothelium-independent vasoconstriction via protein kinase pathway. The causes of these responses are not clear but are independent from vasoactive cell membrane receptors, phosphoinositide turnover, or activation of the cyclooxygenase (Luwaert et al., 1991; Mosseri et al., 1993; Shoemaker et al., 2004; Sudhoff et al., 2004).

However, in contrast to this theory there is no evidence that calcium channel blockers or nitrates reduce the risk of cardiotoxicity (Patel et al., 1987; Eskilsson and Albertsson, 1990). These data suggest that there are other mechanisms of cardiotoxicity.

De Forni et al. (1992) suggested the possibility of a direct drug toxic action on the myocardium, particularly giving evidence that there is global systolic dysfunction, which does not correspond to any individual coronary artery territory.

The intravenous bolus and the continuous administration modalities of 5-FU are both cardiotoxic even if the mechanism of heart toxicity is different. It has to be considered that acute toxicity on bone marrow and gastro-intestinal tract is certainly reduced with the continuous infusion modality (Arkenau et al., 2005). About the cardiotoxicity, Tsibiribi et al. (2006) showed that rabbits receiving bolus 5-FU developed massive hemorrhagic myocardial infarcts with evidence of proximal coronary vasospasm. However, animals treated with repeated low 5-FU doses had heart histologic changes consistent with toxic myocarditis together with the occurrence of apoptosis. These data together with large circadian changes of Css (3–25 times) of intravenous 5-FU could justify the wide variation of cardiotoxicity induced by 5-FU in patients. It has also to be considered that it has been reported an increased incidence of cardiac-related events in patients with continuous 24-h 5-FU infusion for 5 days rather than in patients with short 5-FU administration (Diasio and Harris, 1989; Kosmas et al., 2008; Tsavaris et al., 2005). Therefore, the continuous infusion of 5-FU impacts differently on both cardiotoxicity and general side effects.

In our study, we have shown that 5-FU induced 50% growth inhibition (IC:50) at 72 h with concentrations of 400 μM and 4 μM on H9c2 and HT-29, respectively. Moreover, we have found that the addition of LF to 5-FU potentiated the growth inhibition induced by 5-FU reaching an IC:50 of about 43 μM on H9c2. These concentrations are clinically relevant since the serum levels reached by 5-FU vary from 0.1 to 175 μM (Büchel et al., 2013). In the present manuscript, we have also evaluated in H9c2 and HT-29 cells the effects of 5-FU on intracellular Tbars levels and on the production of NO$_2$$^-$, NO terminal product. We have, indeed, found that 5-FU caused an increase of the peroxidation of membrane lipids with an increase of NO$_2$$^-$ levels. However, cardiocytes were more sensitive to damage induced by pharmacological treatment on peroxidation of membrane lipids compared to colon adenocarcinoma cells probably because of their cancer phenotype and different tissue origins.

All these findings were in agreement with the induction of an increase of the oxidative stress status in cardiocytes. Moreover,

these effects were paralleled with a transition of the mitochondrial potential that could be responsible for apoptosis occurrence as already demonstrated by previous studies (Lamberti et al., 2011).

In fact, although, cardiocytes have protective mechanisms that overcome the apoptotic injury caused by several toxic agents that can circulate in the bloodstream, this program probably is not able to avoid the injury induced by agents with a very high oxidative potential as some anti-cancer agents. This apoptotic effect could be due to the induction of the increase of intracellular ROS in cardiomyocytes exposed to 5-FU $\pm$ LF (Lamberti et al., 2012). The mechanism of cardiomyocytes apoptosis linked to the intracellular oxidative stress are different: (i) generation of free radicals and lipid peroxidation of cardiac membranes (Eken et al., 2010), (ii) myocyte damage induced by cardiac calcium overload (Fucic et al., 1998), (iii) formation of DOX-iron complex (Favier et al., 2003), (iv) impaired myocardial adrenergic regulation, cellular toxicity of anthracycline metabolites (Undeger et al., 1999), and (v) inhibition of beta-oxidation of long chain fatty acids with the consequent depletion of cardiac ATP (Maluf and Erdtmann, 2000).

In conclusion, our data suggest that agents such as 5-FU different from anthracyclines, that are conventionally related to the induction of cardiotoxic effects, can also induce cardiocyte damage that is paralleled by oxidative stress. Therefore, strategies based upon the use of scavengers could be useful in order to prevent this effect. The data obtained in this study will be confirmed in vivo with a series of experiments already in preparation. Moreover, we are performing experiments in order to find molecular markers predictive of cardiac toxicity (such as miRNAs) both in vitro and in a preclinical model of cardiotoxicity.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

References

Arkenau, H.T., Rettig, K., Porschen, R., 2005. Adjuvant chemotherapy in curative resected colon carcinoma: 5-fluorouracil/leucovorin versus high-dose 5-fluorouracil 24-h infusion/leucovorin versus high-dose 5-fluorouracil 24 h infusion. Int. J. Colorectal Dis. 30, 258–261.

Ashraf, N., Hoffe, S., Kim, R., 2013. Adjuvant treatment for gastric cancer: chemotherapy versus radiation. Oncologist 18, 1013–1021.

Aziz, S.A., Tramboo, N.A., Mohi-ud-Din, K., et al., 1998. Supraventricular arrhythmia: a complication of 5-fluorouracil therapy. Clin. Oncol. 10, 377–378.

Brestescher, C., Pautier, P., Farge, D., 1995. Chemotherapy and cardiotoxicity. Ann. Cardiol. Angeiol. 44, 443–447.

Büchel, B., Rhyn, P., Schürch, S., Bühr, et al., 2013. LC–MS/MS method for simultaneous analysis of uracil, 5,6-dihydrouracil, 5-fluorouracil and 5-fluoro-5,6-dihydrouracil in human plasma for therapeutic drug monitoring and toxicity prediction in cancer patients. Biomed. Chromatogr. 27, 7–16.

Burger, A.J., Mannino, S., 1987. 5-fluorouracil-induced vasospasm. Am. Heart J. 114, 433–436.

Caciari, T., Casale, T., Tomei, F., et al., 2012. Exposure to antineoplastic drugs in health care and blood chemistry parameters. Clin. Ter. 163, 387–392.

Caraglia, M., Giuberti, G., Marra, M., et al., 2011. Oxidative stress and ERK1/2 phosphorylation as predictors of outcome in hepatocellular carcinoma patients treated with sorafenib plus octreotide LAR. Cell Death Dis. 2, e150.

Chiosi, E., Spina, A., Sorrentino, A., et al., 2007. Change in TNF-receptor expression is a relevant event in doxorubicin-induced H9c2 cardiomyocyte cell death. J. Interferon Cytokine Res. 27, 589–597.

Connor, T.H., DeBord, D.G., Pretty, J.R., et al., 2010. Evaluation of antineoplastic drug exposure of health care workers at three university-based US cancer centers. J. Occup. Environ. Med. 52, 1019–1027.

De Forni, M., Malet-Martino, M., Jaillais, P., et al., 1992. Cardiotoxicity of high-dose continuous infusion florouracil: a prospective clinical study. J. Clin. Oncol. 10, 1795–1801.

Diasio, R.B., Harris, B.E., 1989. Clinical pharmacology of 5-fluorouracil. Clin. Pharmacokinet. 16, 215–237.

Duschinsky, R., Plevan, E., Heidelberg, C., 1957. The synthesis of 5-fluoropyrimidines. J. Am. Chem. Soc. 79, 4559–4560.

Eken, A., Aydin, A., Erdem, O., et al., 2010. Cytogenetic analysis of peripheral blood lymphocytes of hospital staff occupationally exposed to low doses of ionizing radiation. Toxicol. Ind. Health 26, 273–280.

Eskilsson, J., Albertsson, M., Mercke, C., 1988. Adverse cardiac effects during inductionchemotherapy treatment with cis-platin and 5-fluorouracil. Radiother. Oncol. 13, 41–46.

Eskilsson, J., Albertsson, M., 1990. Failure of preventing 5-fluorouracil cardiotoxicity by prophylactic treatment with verapamil. Acta Oncol. 29, 1001–1003.

Favier, B., Gilles, L., Desage, M., et al., 2003. Analysis of cyclophosphamide in the urine of antineoplastic drugs handlers. Bull. Cancer 90, 905–909.

Fucic, A., Jazbec, A., Mijic, A., et al., 1998. Cytogenetic consequences after occupational exposure to antineoplastic drugs. Mutat. Res. 416, 59–66.

Gomez-Monterrey, I., Campiglia, P., Scognamiglio, I., et al., 2013. DTNQ-Pro, a mimetic dipeptide, sensitizes human colon cancer cells to 5-fluorouracil treatment. J. Amino Acids 2013, 509056.

Gorgulu, S., Celik, S., Tezel, T., 2002. A case of coronary spasm induced by 5-fluorouracil. Acta Cardiol. 57, 381–383.

Kosmas, C., Kallistratos, M., Kopterides, P., et al., 2008. Cardiotoxicity of fluoropyrimidines in different schedules of administration: a prospective study. J. Cancer Res. Clin. Oncol. 134, 75–82.

Labianca, R., Beretta, G., Clerici, M., et al., 1982. Cardiac toxicity of 5-flurouracil: a study of 1083 patients. Tumori 68, 505–510.

Lamberti, M., Caraglia, M., Zappavigna, S., et al., 2011. Evaluation in vitro of the cardiotoxic effects of 5-fluorouracil for the prevention of cardiovascular damage in health workers occupationally exposed. G. Ital. Med. Lav. Ergon. 33, 298–302.

Lamberti, M., Porto, S., Marra, M., et al., 2012. 5-Fluorouracil induces apoptosis in rat cardiocytes through intracellular oxidative stress. J. Exp. Clin. Cancer Res. 31, 60, 19.

Lamberti, M., Giovane, G., Garzillo, E., et al., 2014. Animal models in studies of cardiotoxicity side effects from antiblastic drugs in patients and occupational exposed workers. Biomed. Res. Int. 2014, 8, http://dx.doi.org/10.1155/2014/240642, Article ID 240642.

Luwaert, R., Descamps, O., Majois, F., et al., 1991. Coronary artery spasm induced by 5-flurouracil. Eur. Heart J. 12, 468–470.

Maluf, S.W., Erdtmann, B., 2000. Evaluation of occupational genotoxic risk in a Brazilian hospital. Genet. Mol. Biol. 23, 485–488.

Meyer, C.C., Calis, K.A., Burke, L.B., et al., 1997. Symptomatic cardiotoxicity associated with 5-fluorouracil. Pharmacotherapy 17, 729–736.

Mosseri, M., Fingert, H.J., Varticovski, L., et al., 1993. In vitro evidence that myocardial ischemia resulting from 5-fluorouracil chemotherapy is due to protein kinase C-mediated vasoconstriction of vascular smooth muscle. Cancer Res. 53, 3028–3033.

Patel, B., Kloner, R.A., Ensley, J., et al., 1987. 5-Fluorouracil cardiotoxicity: left ventricular dysfunction and effect of coronary vasodilators. Am. J. Med. Sci. 294, 238–243.

Rezkalla, S., Kloner, R., Ensley, J., et al., 1989. Continuous ambulatory ECG monitoring during flurouracil therapy: a prospective study. J. Clin. Oncol. 7, 509–514.

Rödel, C., Hofheinz, R., Liersch, T., 2012. Rectal cancer: state of the art in 2012. Curr. Opin. Oncol. 24, 441–447.

Shoemaker, L., Arora, U., Rocha Lima, C., 2004. 5-Fluorouracil-induced coronary vasospasm. Cancer Control 11, 46–49.

Sorrentino, M.F., Kim, J., Foderaro, A.E., Truesdell, A.G., 2012. 5-Fluorouracil induced cardiotoxicity: review of the literature. Cardiol. J. 19, 453–458.

Sudhoff, T., Enderle, M., Pahlke, M., et al., 2004. 5-Fluorouracil induces arterial vasocontractions. Ann. Oncol. 15, 661–664.

Teixeira, L., Barry, S., Debourdeau, P., Cohen, A., et al., 2004. Cardiotoxicité du 5-fluoro-uracile. Bull. Cancer 91, 154–158.

Titheradge, A., 1998. The enzymatic measurement of nitrate and nitrite. Methods Mol. Biol. 100, 83–91.

Tsavaris, N., Kosmas, C., Vadiaka, M., et al., 2005. 5-Fluorouracil cardiotoxicity is a rare, dose and schedule-dependent adverse event: a prospective study. J. BUON 10, 205–211.

Tsibiribi, P., Bui-Xuan, C., Bui-Xuan, B., et al., 2006. Cardiac lesions induced by 5-fluorouracil in the rabbit. Hum. Exp. Toxicol. 25, 305–309.

Undeger, U., Basaran, N., Kars, A., et al., 1999. Assessment of DNA damage in nurses handling antineoplastic drugs by the alkaline COMET assay. Mutat. Res. 439, 277–285.

Weidmann, B., JansenW, Heider, A., et al., 1995. 5-Fluorouracil cardiotoxicity with left ventricular dysfunction under different dosing regimens. Am. J. Cardiol. 75, 194–195.

Yoshida, J., Koda, S., Nishida, S., et al., 2011. Association between occupational exposure levels of antineoplastic drugs and work environment in five hospitals in Japan. J. Oncol. Pharm. Pract. 17, 29–38.