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COORDINATOR: PROF. EMANUELE ALBANO

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STUDENT: **SILVIA ANTONINI** CYCLE: **XXIX** YEAR: **2014-2015**

TUTOR: PROF. MARIA GIOVANNA PRAT

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1. First Year Results Summary

Cardiovascular diseases are the leading cause of morbidity and mortality in the industrialized countries (Gräsner and Bossaert, 2013). Several studies have demonstrated that apoptosis is involved in both acute and chronic cases and it is one of the prevalent phenomenon during myocardial infarction, This data suggests an important role of apoptosis during the acute phase of heart attack. Moreover, apoptotic phenomenons have been shown during later phase suggesting an involvement in the heart remodeling. Short term treatment with H_2O_2 induces apoptosis in H9c2 cardiomyoblasts, as already reported (Han et al., 2004). This observation is confirmed by the acquisition of features typical of this event, namely, positivity to annexin V, activation of caspases, and fragmentation of genomic DNA. This treatment induces also an increase in the cellular levels of ROS, thus establishing a cause–effect relationship in promoting apoptosis. Similar data were reported also in the case of oxidative stress induced in these cells by other molecules. For the first time, we demonstrated that clovamide, a minor constituent of cocoa, is able to protect cardiomyoblasts from apoptosis induced by oxidative stress by counteracting the effects induced by ROS. We have shown that clovamide, as well as its isostere (rosmarinic acid) and the main monomeric flavan-3-ol from cocoa ((-)-epicatechin) interfere with and reduce apoptosis triggered by the H_2O_2 treatment in H9c2 cardiomyoblasts by decreasing the levels of intracellular ROS. The analyses for annexin V positivity, DNA fragmentation, and caspase activation and release provided significant evidence on the direct protective efficacy of clovamide. This work, thus, confirms and strengthens the radical scavenging properties of these polyphenols and their anti-apoptotic activity. These data will be the basis for similar experiments to be performed on human cardiac progenitor cells (hCPCs), which, being cells obtained from patients undergoing cardiac surgery, are more difficult and time-consuming to be prepared. Other natural compounds, such as curcumin, α-bisabolol, quercetin and rice extracts, will also be tested in this context for their potential protective effects on H_2O_2-induced apoptosis.

2. Background

Mesenchymal stem cells (MSCs) are a promising tool to improve tissue repair. The administration of these cells was shown to be able to contribute to the regeneration of different tissues in organs such as, for example, heart, kidney and lung, although with different efficacy in different cell types (Monsel et al., 2014).

They may be involved directly to repair damaged tissue, but also, and perhaps, more frequently, they may have a beneficial role through a paracrine mechanism, by releasing soluble factors with pro-mitotic, anti-apoptotic, anti-inflammatory and immunomodulatory functions. Moreover, they can regulate the imbalances induced by oxidative stress. Free oxygen radicals or, more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cellular metabolism. They are highly reactive molecules and they include superoxide anion (O_2-), hydrogen peroxide (H_2O_2), hydroxide (OH-), hypochlorite (OCl^-), nitroxyl anions, nitrosonium cation, higher oxides of nitrogen, S-nitrosothiols, and dinitrosyl iron complexes. ROS and RNS are well recognised for playing a dual role both as detrimental and beneficial species, since they can be either harmful or beneficial for living systems (Valko et al., 2006). Beneficial effects of ROS occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia, in defence against infectious agents and in the function of a number of cellular signalling systems (Valko et al., 2007). The damaging effect of free radicals occurs in biological systems when there is an imbalance between the production of ROS/RNS and the antioxidants systems. In fact, at high concentrations, ROS can be important mediators of damage to cell macromolecules, such as nucleic acids, lipids and proteins.

Furthermore, some ROS act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruption in normal mechanisms of cellular signaling.
Defense mechanisms against free-radical induced oxidative stress involve enzymatic antioxidants (dismutase, glutathione peroxidase, catalase) and non-enzymatic antioxidants (ascorbic acid, α-tocopherol, glutathione, carotenoids, flavonoids). In humans, oxidative stress is thought to be involved in the development of cancer (Van de Bittner et al., 2010), neurodegenerative diseases (Patel and Chu, 2011), cardiovascular diseases, diabetes, ischemia/reperfusion damage (Dalle-Donne et al., 2006), heart failure, myocardial infarction (Singh et al.), other diseases and in ageing.
One of the main problems in the use of MSCs in vivo is their difficulty to survive and proliferate in the damaged tissue that represents a hostile microenvironment, since ROS affect cells' functions, triggers apoptosis and may lead to the failure of the engraftment (Grossmann, 2002; Ingber, 2002). It is therefore essential to prevent oxidative stress-induced apoptosis in transplanted cells. The pre-conditioning of cells before transplantation by means of quick induction of oxidative stress (pre-conditioning) or by pre-treatment with antioxidants might be a good strategy to protect the transplanted cells in a hostile site and to increase engraftment/survival thanks to the induction of cytoprotective pathways (Yagi et al., 2013). In the last years antioxidants from natural origin have received an increasing attention. Among polyphenols, curcumin, the main constituent of the turmeric spice, has attracted a lot of attention due to its ability to exert beneficial effects in multiple pathological conditions. Curcumin was shown to exert a potent scavenger activity for a variety of ROS such as O2-, OH-, nitrogen dioxide radicals and non-free radical species such as H_2O_2. It was also shown to enhance the activity of antioxidant enzymes and, in particular, to counteract the activity of ROS generating enzymes. Since increased oxidative stress is associated to various diseases, the inhibitory activity of curcumin on ROS generation coupled with its anti-inflammatory properties may contribute to its protective role (Kapakos et al., 2012).
In our study we used a spontaneously immortalized cell line, named m17.ASC, that was derived from adipose tissue of adult FVB/N mice (Zamperone et al., 2013). This stable and not tumorigenic cell line was used to investigate possible pharmacological modulations induced by curcumin and its molecular mechanism of action in cytoprotection.

3. Project aim/objectives

In the laboratory of Histology we previously showed that a short-term H_2O_2 treatment leads to oxidative stress and induces apoptosis in H9c2 cells, and that a monoclonal antibody, agonist of the Hepatocyte Growth Factor receptor, could reverse this effect (Pietronave et al., 2010). We want now to extend this kind of studies to molecules from the vegetable world, which are known to display antioxidant and radical scavenging activities, such as clovamide, curcumin, α-bisabolol and black and red rice extracts. In particular we wanted to analyze their ability to inhibit the production and release of ROS and to protect cells from oxidative stress-induced apoptosis. To this purpose, we have used the m17.ASC cells, which represent a good model of spontaneously immortalized adult mesenchymal stem cells (Zamperone et al., 2013). Once the experimental conditions are settled, we will use this information to carry on experiments on human Cardiac Progenitors Cells (hCPCs), obtained from biopsies provided by the Department of Cardiac Surgery from the "Clinica San Gaudenzio" (Novara, Italy) and obtained from patients undergoing cardiac surgery. All the patients involved will sign a written informed consent according to a protocol approved by the Institutional Review Board (IRB) of Novara (Italy). On all these cells, we will evaluate the protective effects of the different pure polyphenols and plants extracts on the H_2O_2-induced oxidative stress, using different tests. Moreover, aim of this project is also to establish pathways and mechanisms of action involved in these cell biological responses using Real-time PCR and Western Blot analysis. In my second year of PhD

program I have examined if curcumin could exert beneficial activities on MSCs, when they are exposed to oxidative stress. We used two different schemes of treatment, where cells were treated with curcumin before (pre-conditioning) and after the oxidative insult. m17.ASC were treated with different concentrations of curcumin ranging from 0.5 to 5 μM, and challenged with increasing concentrations of H_2O_2 (0–1000 μM) to mimic oxidative stress conditions. We analyzed how preconditioning or post stress treatment with curcumin affected overall viability of the cells or regulated the expression of genes and proteins involved in the apoptotic pathway.

4. Experimental plan and methods

CHEMICALS

Curcumin, gelatin, dimethyl sulfoxide (DMSO), ethylenediaminetetracetic acid (EDTA), Claycomb Medium, 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT), QuantiPro™ BCA Assay Kit were purchased from Sigma Aldrich (St. Louis, MO, U.S.A.). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin and L-Glutamine were from Lonza Group Ltd (Verviers, Belgium). The tissue culture plates were from Orange Scientific (Braine-l'Alleud, Belgium). Water was obtained by Milli-Q instrument (Millipore Corp., Billerica, MA). Coversplips were purchased from Marienfeld (Lauda-Konigshofen, Germany). Annexin V-FITC and Propidium iodide were from Alexis (Lausen, Switzerland). Primary antibody against Active Caspase-9 and β-Tubulin was purchased from Cell Signaling. Primary antibody against Active Caspase-3 was from Alexis Biochemicals. HRP-Conjugated secondary antibodies were from Amersham. For RT-PCR Trizol® reagent was obtained from Invitrogen, DNAse I and RevertAid™ H Minus First Strand cDNA Synthesis Kit were from Fermentas. To perform Quantitative Real-Time PCR was used the SsoFast™ EvaGreen® supermix, obtained from Bio-Rad.

CELL CULTURE

m17.ASC clone was obtained in our laboratory as described by Zamperone and colleagues (Zamperone et al., 2013). Cells were cultured in Claycomb Medium containing 1500 mg/l $NaHCO_2$, 10% FBS, 50 U per ml penicillin and 50 μg/ml streptomycin. Cells were usually passaged and used for the experiments when 80% confluent.

INDUCTION OF OXIDATIVE STRESS

Oxidative stress was induced as described by Pietronave and colleagues (Pietronave et al., 2010) with few modifications. Cells were washed twice in PBS and then incubated in serum-free medium containing different concentrations of H_2O_2; the medium was then changed and cells were incubated in presence or not of different concentrations of curcumin from 3 to 24 hours depending on the assay.

MTT

m17.ASC cells (10^4 cells/well in 96- well plates) were incubated for 24 h, and then different concentrations of curcumin, ranging from 0.05 to 50 μM, or different concentrations of H_2O_2 were added in 100 μL of fresh medium. After one-day incubation, cell viability was evaluated by MTT colorimetric assay. Briefly, 20 μL of MTT solution (5 mg/ml in a PBS solution) was added to each well. The plate was then incubated at 37 °C for 3 h. After the removal of the solution, 125 μL of isopropanol 0.2 N HCl was added to dissolve formazan crystals, 100 μL were carefully recovered and their optical density was measured in a multiwell reader (2030 Multilabel Reader Victor TM X4, PerkinElmer) at 570 nm. Viability of parallel cultures of untreated cells was taken as 100% viability,

and values obtained from cells undergoing the different treatments were referred to this value. Experiments were performed 4 times using 3 replicates for each sample.

ANNEXIN V / PROPIDIUM IODIDE

At least 50,000 cells, induced to apoptosis by the above described H_2O_2 treatments, were incubated or not with curcumin for 24 hours as described. Cells were then detached with 5 mM EDTA, washed twice with PBS and incubated for 15 minutes at room temperature with Annexin V – FITC (100 nm) and Propidium Iodide (50 µg/ml) both diluted in a Hepes/NaOH 10 mM, NaCl 140 mM, CaCl2 2,5 mM (pH 7.4) buffer. Cells were then washed twice with ice cold PBS and fixed in buffered 1% paraformaldehyde, 2% FBS for 15 minutes at 4°C and analyzed with FACScan flow cytometer (Becton Dickinson, Mountain View, CA) equipped with a 488-nm argon laser.

WESTERN-BLOT

Subconfluent cells were induced to oxidative stress as described above. Cells were washed twice with cold phosphate-buffered saline (PBS) and lysed in RIPA Buffer (Hepes 500 mM, pH 7,4, 5 mM NaCl, 10 %, SDS 1% Triton X 100, 10% deoxicolic acid, 190 mM $MgCl_2$, 500 mM EGTA, 500 mM NaF, 1/1000 Leupeptin, 1/1000 Pepstatin, 1/100 PMSF). Cell lysates were centrifuged at 13,000 rpm and 4°C for 15 min. Protein concentration of the lysates was evaluated with QuantiPro™ BCA Assay Kit and 50 µg of proteins of each sample was denatured by heating for 5 min at 95°C in reducing Laemmli buffer; proteins were resolved on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were then transferred onto PVDF filters, which were blocked with methanol for 5 min, rinsed in water, and probed either with mAbs against Active Caspase-9 and Active Caspase-3 diluted in Tris-buffered saline (TBS)-5% BSA over-night at 4°C. After extensive washing, immunocomplexes were detected with horseradish peroxidase-conjugated secondary antibodies followed by enhanced chemiluminescence.

RT-PCR AND QUANTITATIVE REAL-TIME PCR

For reverse transcription-PCR (RT-PCR), total RNA was extracted in the Trizol® reagent, followed by DNAse I treatment. Then, 1 µg of RNA was retrotranscribed into cDNA with the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas). Quantitative Real-Time PCR was performed using the SsoFast™ EvaGreen® supermix, using the using the oligo(dT) primers listed in *Table 1*. The PCR protocol, using the CFX96® Real-Time System (Bio-Rad), was: 96 °C for 1 minute, 45 cycles 95 °C for 5 seconds, annealing and extension at 60 °C for 5 seconds. To analyze data was used the software CFX Manager®.

STATISTICAL ANALYSIS

Quantitative analysis are presented as mean + standard deviation (SD) and differences between samples were determined by Student's t-test. One-way ANOVA and Bonferroni post-test analyses on selected pairs of groups were also performed with Prism (GrapPad software Inc., USA, version 4.03). Values with a $p<0.05$ or $p<0.01$ were considered as statistically significant.

GENE		SEQUENCE	TM (°C)	AMPLIFICATE LENGTH
Bax	F	5'-GACACCTGAGCTGACCTTGGA-3'	72,8	200
	R	5'-GACACTCGCTCAGCTTCTTGGT-3'		
CDKN1A (p21)	F	5'-GTCCCACTTTGCCAGCAGAATAA-3'	71,8	66
	R	5'-GGTCGGACATCACCAGGATTG-3'		
GADD45A	F	5'-CCTGCACTGTGTGCTGGTGA-3'	64	106
	R	5'-CCACTGATCCATGTAGCGACTTTC-3'		
p53	F	5'-TTGGACCCTGGCACCTACAATG-3'	73,2	125
	R	5'-GCAGACAGGCTTTGCAGAATGG-3'		
Nrf2	F	5'-GAACTGTAGGAAAAGGAAGC-3'	58	210
	R	5'-GAGTATTCACTGGGAGAGTA-3'		
NQO1 (p32)	F	5'-AGCCCCACCAAGTTCAAACA-3'	60	235
	R	5'-TCTCTGCAGGGGCAGTATCT-3'		
SOD1	F	5'-GTGAACAATCTCAACGCCAC-3'	60	185
	R	5'-GCCTCCAGCAACTCTCCTTT-3'		
GAPDH	F	5'-CGTGGATCTGACGTGCCGCC-3'	68	250
	R	5'-CACCACCCTGTTGCTGTAGC-3'		

Table 1: Primers sequences used in Quantitative Real-time PCR

5. Results

CELL VIABILITY AFTER THE TREATMENT WITH CURCUMIN AND H_2O_2

Oxidative stress was performed on m17.ASC cells with the protocol of Zamperone and colleagues (Zamperone et al., 2014) with slight modifications. Preliminary experiments were performed to determine the effects on cell viability after the incubation with different concentrations of curcumin and H_2O_2, separately. Thereafter, cell viability was assessed with MTT assay. Cells were exposed to H_2O_2 for a short-term treatment (15 min) and then incubated for a further 24 h in complete medium or treated for 24 h with curcumin. As shown in the figure H_2O_2 caused a dose-dependent reduction of cell viability (*Fig. 1 A*). Curcumin concentrations below 5 μM have no effect on cell viability, while increasing concentration show a negative effect on cell proliferation (*Fig. 1B*). Cells were then treated with both curcumin and hydrogen peroxide. We used two different schemes of treatment, where cells were treated with curcumin before (pre-conditioning) (*Fig. 1C*) or after (*Fig. 1D*) the oxidative insult. Curcumin exerted an significant protective effect against stress insult, since it preserved the viability and proliferative potential, in a dose-dependent manner, but only when these were pre-conditioned (24 hours of continuous exposure). If curcumin was administered after H_2O_2 challenge, no significant protection was observed at any concentration.

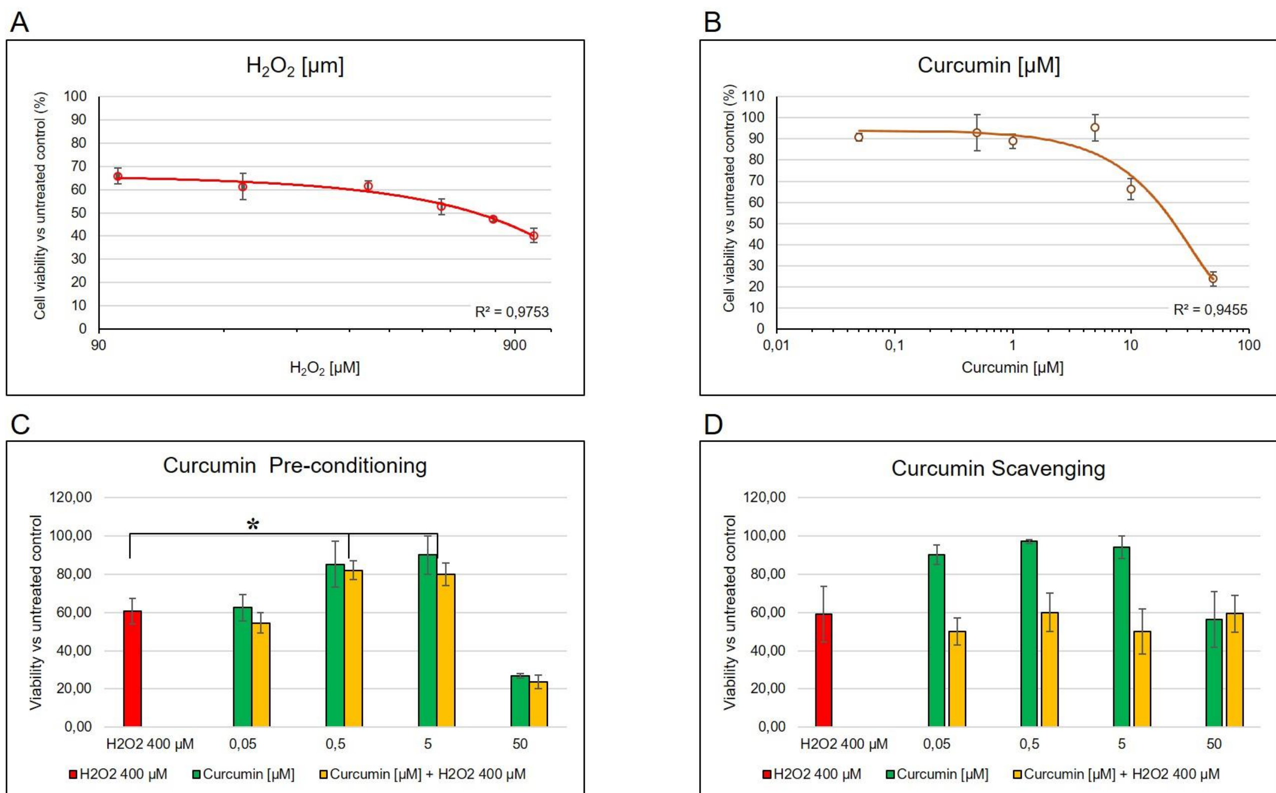


Figure 1: Cell viability after the treatment with Curcumin and H_2O_2 (MTT Assay): m17.ASC cells were incubated with different concentrations of H_2O_2 (A) or Curcumin (B) for 24 hours. H_2O_2 caused a dose-dependent reduction of cell viability concentrations. Curcumin below 5 μM have no effect on cell viability, while increasing concentration show a negative effect on cell proliferation. B) and C) The effects of different schedulings are shown. Cell monolayers were pre-treated (pre-conditioning) 24 h (C) before or (D) after the challenge with H_2O_2 to mimic oxidative stress. Under these conditions only the pre-conditioning was able to elicit an effective protective effect. Significance was determined by Dunnet post-test* $p<0,001$

EFFECTS OF CURCUMIN ON H_2O_2 – INDUCED APOPTOSIS

Cells were pre-conditioned with Curcumin for 24 h and challenged with H_2O_2 for 15 min. After the treatment with 400 µM H_2O_2, changes in cell morphology were observed, such as cell shrinkage and membrane blebbings,. No significant changes in morphology were detected upon treatment with Curcumin alone or when cells were treated with Curcumin before inducing oxidative stress (*Fig. 2 A*). Annexin V-FITC/PI flow cytometry was used to detect m17.ASCs apoptosis. The percentage of apoptotic cells after treatment with H_2O_2 was triplicated compared to the control. Curcumin, added at a 0.5 and 5 µM concentration, was able to reverse the pro-apoptotic effect of H_2O_2 at a significant level ($p \leq 0.001$) (*Fig. 2 B, C*), as shown in a representative cytogram.

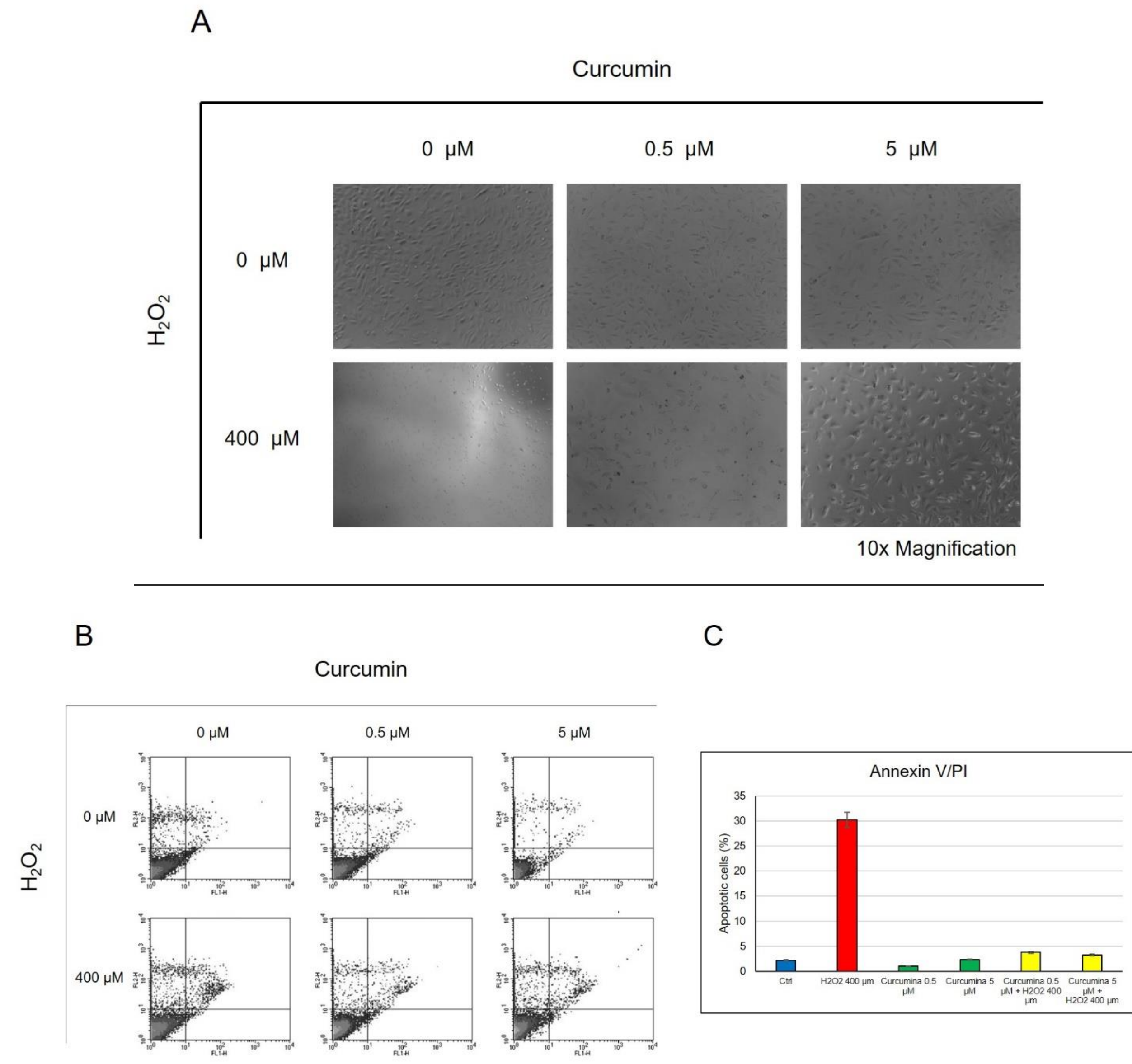
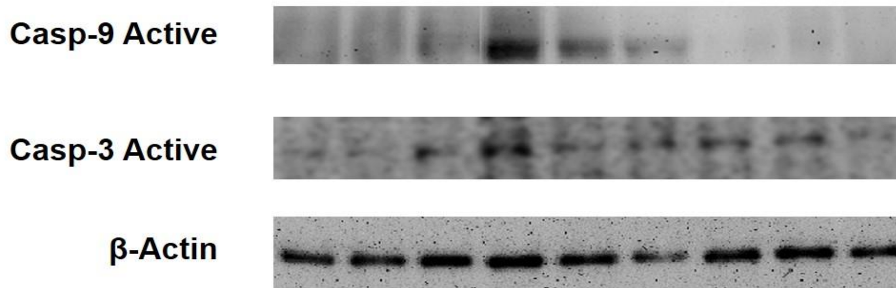


Figure 2: Curcumin protects m17.ASC against apoptosis induced by oxidative stress. A) Representative morphological changes of the treated cells. Cells were incubated for 24 h with curcumin, 15 min with 400 µM H_2O_2 in serum-free medium and, after its withdrawal, for a further 24 h in complete medium. B) Representative cytograms of 1 of the 3 flow cytofluorometric analysis performed. Cell death was monitored after labeling with fluoresceinated annexin V and propidium iodide (PI). C) Graphical representation of the percentage of apoptotic cells. Anova and Bonferroni post-test were performed by comparing cells treated with protective agent vs. untreated cells (* $p < 0.001$

CURCUMIN INHIBITS THE ACTIVATION OF CASPASES INDUCED BY HYDROGEN PEROXIDE TREATMENT

Apoptosis usually results from the activation of caspases by the offending factor. We thus investigated the ability of clovamide to inhibit caspases 3 and 9 activation in m17.ASC cells 2 and 24 h after H_2O_2 withdrawal by Western Blot. H_2O_2 significantly increased the active form of this enzymes, while curcumin reverted this effect.



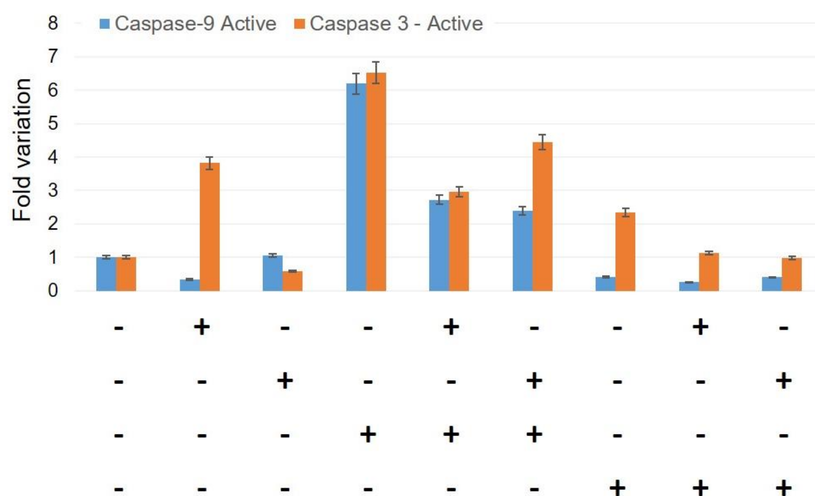


Figure 3: Effect of Curcumin on H_2O_2 induced caspase-9 and caspase-3 activation. Cells were pre-conditioned with Curcumin for 24 h and challenged with H_2O_2 for 15 min. After 2 or 24 h cells were lysated with RIPA. A) representative western blot of the proteins under the different conditions indicated. B) graphical representation of the fold variations of their expression. Anova and Bonferroni post-test were performed by comparing cells treated with protective agent vs. untreated cells (* $p < 0.001$).

CURCUMIN REGULATES THE EXPRESSION OF DIFFERENT GENES INVOLVED IN APOPTOSIS AND OXIDATIVE STRESS

The experimental data obtained by qPCR, although still partial, are reported in Figure 4. We analyzed factors involved in the apoptotic pathway such as Bax, p53, p21, or in the mechanisms of cell detoxification such as Nrf2, NQO1 and SOD1. Data are expressed as ratio of expression relative to untreated cells, after normalization to the housekeeping gene, GAPDH. The analysis of the transcriptional levels of p53 demonstrated a transcriptional reduction after the treatment with curcumin compared with H_2O_2, although they are not significant due to a low basal expression of this gene. Bax expression levels did not change after the treatments performed. The effects of the pretreatment with curcumin on in the Bax expression was evident only at a concentration of 5 uM, where a significant decrease in its expression compared to the hydrogen peroxide treatment was observed. The analysis of the expression levels of p21, whose transcription is induced by p53, demonstrated that only the concentration of 5 uM was able to down-regulate significantly its expression. The variation of the expression levels of GADD45, the product of activation of p53, show an induction in the positive direction by curcumin at the concentration of 0.5 uM with values similar to those induced by peroxide. Treatment with 5 uM Curcumin did not induce a significant transcription of this product while it was able to inhibit its transcription significantly after the treatment with H_2O_2. The treatment with curcumin 0.5 uM was able to increase significantly the expression of Nrf2, a transcription factor responsible for the regulation of the expression of antioxidant and detoxifying enzymes such as NQO1 and SOD. This dose of curcumin was able to induce a significant effect on the Nrf2 transcription, higher than the same peroxide. The preconditioning with this concentration of curcumin did not modify the effects induced by hydrogen peroxide, while the concentration of 5 uM was able to significantly reduce the increase of Nrf2 transcription. SOD1, does not seem to be significantly modified in its expression as a consequence of the different treatments.

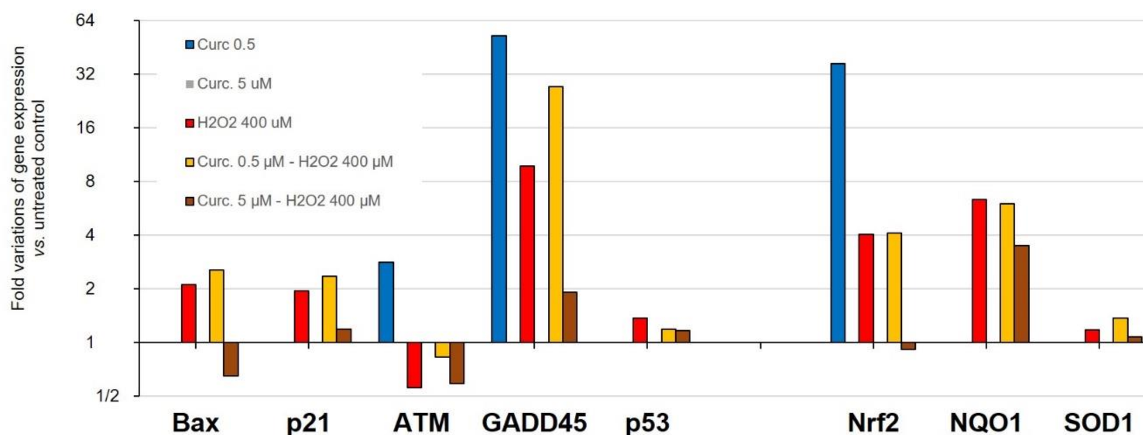


Figure 4: Gene expression of different genes in apoptosis and oxidative stress. Data are expressed as mean fold variations of gene expression in cells pre-conditioned with Curcumin 0,5 μM and 5 μM and than challenged with H_2O_2.

6. Discussion

MSCs are one of the most attractive choices in cell therapy. One of the main problems of their use in vivo is their difficulty to survive and proliferate in the damaged tissue. One of the factors that contribute mainly to the loss of cells transplanted is oxidative stress (Grossmann, 2002). It is therefore essential to prevent apoptosis in the injected cells. The pre-conditioning of the cells before transplantation or their pre-treatment with antioxidants might be a good strategy to protect them and to increase their engraftment and survival. In our study we used H_2O_2 to mimic the tissue hostile microenvironment often found when transplanting. For our experiment we choose the spontaneously immortalized cell line m17.ASC, isolated and characterized in laboratory since it is a useful model of mesenchymal stem cell line (Zamperone et al., 2013). We showed that, after a short treatment with increasing concentrations of hydrogen peroxide, cell viability decreases in a dose dependent manner, as verified by MTT assay. Similarly, the treatment with hydrogen peroxide at a final concentration of 400 µM for 15 minutes, determined an increased apoptosis, as shown with Annexin V/Propidium Iodide assay. This treatment also leads to the activation of Caspase 3 and 9, as shown by Western Blot analysis. After defining the conditions useful to induce cell death with hydrogen peroxide, we demonstrated the protective potential of curcumin, the main biologically active component of Tumerico, used at different concentrations on m17.ASC treated with hydrogen peroxide. It was previously shown that curcumin is able to reduce the damage induced by oxidative stress in endothelial cells and that it can protect myocardium from ischemia/reperfusion damage by attenuating oxidative stress and mitochondrial dysfunction (González-Salazar et al., 2011). To this purpose we used two experimental protocols aimed to understand the mechanism by which curcumin acts. In the first protocol cells were treated for 24 hours with curcumin before inducing oxidative stress (pre-conditioning), while the second protocol provided first the induction of oxidative stress with hydrogen peroxide and later incubation with curcumin (scavenging). MTT assay showed that only pre-treatment of m17.ASC with curcumin it is able to preserve the viability, which was reduced after the treatment with hydrogen peroxide. By contrast, the scavenging protocol was ineffective. Based on this result, we decided to conduct further experiments using only the pre-conditioning protocol. Curcumin pre-conditioning leads to inhibition of apoptosis induced by hydrogen peroxide and inhibit caspases 3 and 9 activation. We used quantitative Real Time PCR to analyse the modulation of the expression of genes involved in apoptosis and oxidative stress response. These data, although preliminary, indicate that Bax it is not the mechanism by which curcumin, at these concentration, exerts its effects. A Bax reduction, albeit insignificant, was observed in the case of pretreatment with 5 µM curcumin, that may indicate that there are different mechanisms of action attributable to this molecule. A reduction of the expression of Bax, and therefore probably also of its activation, could contribute to the reduction of the proapoptotic mitochondrial pathway. This is partially supported by the levels of expression of p21, a transcriptional product of p53 activation, since only at the concentration of 5 µM, curcumin has been shown to significantly down-regulate its expression. Interesting, albeit difficult to interpret, is the variation of expression levels of GADD45, also produced as a result of the activation of p53. The transcriptional levels were increased with 0,5 µM curcumin and the values were similar to those induced by hydrogen peroxide. The concentration of 5 µM did not induce significant transcription, while it was able to inhibit its transcription significantly after the treatment with H_2O_2. After the treatment with hydrogen peroxide was observed a significant increase in the expression of Nrf2, a transcription factor normally associated with the cytoskeleton but whose activation and induction of transcription leads to transcription of a number of factors such as NO1, NAD(P)H, NQO1. Curcumin, used at the concentration of 0,5 µM, seems to be able to induce a significant effect on Nrf2 transcription, even superior to H_2O_2. Pre-conditioning with curcumin 5 µM

was able to reduce the transcriptional activity of Nrf2. SOD1, does not seem to be modified significantly in its expression under these experimental conditions. These data confirm that the dose of curcumin and the scheduling of treatment adopted affects the response of m17.ASC cells. Further experiments will be conducted to confirm and extend these first experimental observations. These results allow us to say that curcumin is able to protect cells m17.ASC from oxidative stress. In the future, this molecule could be used for in vivo studies aimed at evaluate the effectiveness in enhancing the survival and engraftment of transplanted cells into damaged tissues such as, for example, the heart.

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8. Publications (articles, posters, abstracts)

- **<u>Posters</u>**

- *A pharmacological role of curcumin in the protection from oxidative stress of mesenchymal stem cells of murine origin*
  <u>S. Antonini</u>[1], M. Prat[1], I. Viano and D. Colangelo[1]
  Congresso nazionale SIF – Napoli dal 27 al 30 ottobre 2015

- *Clovamide and curcumin protect mesenchymal stem cells from ROS-induced injury*
  <u>S. Antonini</u>, D. Colangelo, M. Arlorio, J. D. Coisson, I. Viano and M. Prat
  International Spring Research Day – 19th June 2015 – Villa Negroni – Vezia (Lugano)
  <u>Best poster award</u>

9. Attended lessons and seminars

- **Lessons**

- "Tissue engineering: the state of the art" – 14 November 2014 – Dott.ssa Francesca Boccafoschi - Department of Health Sciences, University of Eastern Piedmont.
- "Regenerative Medicine" – 21 November 2014 – Prof. Maria Prat - Department of Health Sciences, University of Eastern Piedmont.
- "Ribosomopathies" – 25th May 2015 – Prof. Steve Ellis – Medical School, University of Louisville (Kentucky)
- "Basis of scientific research" – 10th June 2015 – Dott.ssa Nicoletta Filigheddu – Università del Piemonte Orientale (Italy)

- **Seminars**

- "Dysregulated antigen receptor signaling: molecular lessons from two congenital lymphoproliferative disorders" – 06 November 2014 - Prof. Andrew L. Snow - Department of Pharmacology Uniformed Services University of the Health Sciences Bethesda (Maryland, USA).
- "Optical coherence tomography from bench to bedside shening the light during percutaneous vascular intervention" – 17 November 2014 - Dott. Secco Gioel Gabrio – Department of Health Sciences, University of Eastern Piedmont.
- "La scoperta del bosone di Higgs" – 25 November 2014 - Dott. Roberta Arcidiacono - DiSCAFF, University of Eastern Piedmont - Dott. Marta Ruspa - Department of Health Sciences, University of Eastern Piedmont.

- “Nuove sfide ed opportunità dell'epidemiologia molecolare per lo studio dei tumori” – 27 November 2014 - Prof. Laura Baglietto - Inserm - Centre for Research in Epidemiology and Population Health, Unit: Nutrition, Hormones and Women’s Health, Paris.
- “Humoral responses to HCV infection and clinical outcomes” – 28 November 2014 - Dott. Arvind Patel - Programme Leader, MRC Centre for Virus Research, University of Glasgow (UK).
- “Microglia microvescicles: messengers from the diseased brain” – 17 December 2014 - Dott. Roberto Furlan, San Raffaele University, Milan.
- “Anticancer strategy Targeting cancer cell metabolism in ovarian cancer” – 19 January 2015 - Prof. Dr Yong-Sang Song, MD, PhD Director Cancer Research Institute, Gynecologic Oncology Chariman, Cancer Biology Interdisciplinary Program Professor, Obstetrics and Gynecology, College of Medicine Seoul National University.
- “Different molecular mechanisms regulate hepatocyte differentiation during the transitions between epithelial and mesenchymal states” – 20 January 2015 - Dott. Tonino Alonzi, PhD, Lab. Of Gene Expression and Experimental Hepatology, Istituto Nazionale per le Malattie Infettive “L. Spallanzani” IRCCS, Rome.
- “Targeting the liver to cure myocarditis: a lesson from a model of STAT3-dependent auto-immune myocarditis” – 21 January 2015 - Prof. Valeria Poli - Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Turin.
- “Myeloid cells as therapeutic target in cancer” – 27 January 2015 - Prof. Antonio Sica - DiSCAFF, UPO, Novara.
- “Proof of principle for cell therapy: from autologous transplantation of tissue specific progenitors to gene corrected patient specific injured pluripotent stem cells” – 11 March 2015 – Prof. Darko Bosnakovski - Associate Professor, University "Goce Delcev" Stip, Faculty of Medical Sciences, Krste Misirkov bb, 2000 Stip R. Macedonia.
- “Signal control in iNKT cell development and function” – 09 April 2015 - Prof. Xiaoping Zhong, MD, PhD - Associate Professor, Department of Pediatrics-Allergy and Immunology Duke University, Medical Center, Durham (North Carolina, USA).
- “Actin-based mechanisms in the control of gene expression and cell fate” – 21st April 2015 – Prof. Piergiorgio Percipalle – Associate Professor, Department of Cell and Molecular Biology, Karolinska Institutet (Solns, Sweden).
- “An integrated approach to the diagnosis and treatment of ovarian cancer” – 7th May 2015 – Prof. John McDonald, MD, PhD – Integrated Cancer Research Center, School of Biology and Parker H. Petit Institute of Bioengineering and Biosciience, Georgia Institute of Technology, Georgia Tech University, Georgia (Atlanta, USA).
- “Conflicting interests and scientific communication” – 14th May 2015 – Prof. Kathleen Ruff – RightOnCanada Founder, Senior Advisor to the Rideau Institute (Ottawa, Canada).
- “Recent developments in (cutaneous) Human Polyomavirus research” – 5th June 2015 – Mariet C.W. Feltkamp – Associate Professor of Medical Virology, Department of Medical Microbiology, Leiden University Medical Center (Leiden, The Netherlands).
- “High-tech product preservation and operator protection: two apparently opposite requirements in different fields of medicine and biotechnology: the emerging glove box approach” – 15th July 2015 - Dr. Ing. Marco Fatta, Phd – COMECER Group (Italy).

- “Le cellule staminali nel danno renale acuto e nel trapianto di rene” – 28th July 2015 - Dr. Vincenzo Cantaluppi, MD – Facoltà di Medicina e Chirurgia, Università di Torino (Italy).
- “Cell based models for studying molecular mechanisms of Facioscapulohumeral Muscolar Distrophy (FSHD)” , “Toward animal model for Facioscapulohumeral Muscolar Distrophy (FSHD)” – 3rd September 2015 - Prof. Darko Boshnakovski, PhD – University Goce Delcev Stip, Faculty of Medical Sciences (Stip, R. Macedonia).
- Miniworkshop on “Biotechnology for Dermatology” – 9th July 2015 - Dr Gwenaël ROLIN, PhD - Clinical Research Engineer - Thomas LIHOREAU - Ingénieur hospitalier, Research and Studies Center on the Integument (CERT), Department of Dermatology, Clinical Investigation Center (CIC INSERM 1431), Besançon University Hospital; INSERM UMR1098, FED4234 IBCT, University of FrancheComté, Besançon, France.