Int. J. Morphol.,
32(1):61-69, 2014.

Cytotoxic Effect of Double Emulsion (W/O/W) $CuSO_4$ Loaded PLA Nanoparticles on MKN-45 Gastric Adenocarcinoma Cell Line

Efecto Citotóxico de Nanopartículas de PLA de Doble Emulsión (W/O/W) Cargadas con $CuSO_4$ sobre la Línea Célular de Adenocarcinoma Gástrico MKN-45

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MONTIEL-EULEFI, E.; JARA, F.; TORO, C.; GARCÉS, M. & LEAL, P. Cytotoxic effect of double emulsion (w/o/w) $CuSO_4$-loaded PLA nanoparticles on MKN-45 gastric adenocarcinoma cell line. ***Int. J. Morphol., 32(1)***:61-69, 2014.

SUMMARY: Poly (L-lactic acid) (PLA) nanoparticles have the approval of the main institutions for drugs administration and therapeutics. However, the use of lactic acid polymer is controversial because lactic acid has been proposed as an energy source for cancer cells. The aim of this study was to evaluate the cytotoxic, apoptotic and cell cycle properties of PLA and $CuSO_4$-loaded PLA biodegradable nanoparticles on MKN-45 gastric adenocarcinoma cell line. PLA nanoparticles for the delivery of the anticancer active principle $CuSO_4$ were obtained using the double emulsion method. PLA and $CuSO_4$ loaded PLA nanoparticles were morphologically characterized and their size determined using transmission electron microscopy (TEM). The cytotoxicity of this drug delivery system was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay; apoptosis was evaluated using YO-PRO-1/Propidium Iodide and cell cycle analysis throughout flow cytometry. $CuSO_4$-loaded PLA nanoparticles were effective inhibitors of MKN45 cancer cell growth. They increased cytotoxicity and apoptosis, and induced G1/Go cell cycle arrest; whereas the anticancer activity was increased using a 96 h treatment of a minimal (1mM) concentration of $CuSO_4$ loaded in 40 μM PLA nanoparticles. The treatment with 40 μM lactic acid and PLA (40 μM) did not increase the rate of cell survival assays related to the control, which indicate that PLA use as a polymer carrier not induce proliferation of MKN-45 cancer cells. Our research presents novel data about the effect of PLA nanoparticles and $CuSO_4$ on gastric cancer cell line MKN45.

KEY WORDS: Copper loaded nanoparticles; Poly-lactic acid; Adenocarcinoma MKN45 cells; Cytotoxicity; Cell cycle.

INTRODUCTION

Gastric cancer is the leading cause of death from malignancy for both sexes, with a mortality rate of around 20 per 100,000 people worldwide. The MKN-45 cell line is a representative model obtained from human gastric cancer, characterized by high levels of metastasis (Takikawa *et al*., 2006). Invasion and metastasis are the main biological characteristics of malignant tumors, and also important factors contributing to the death of gastric cancer patients and affecting the efficacy of their treatment. Gastric cancer is the most common malignancy in Southeast Asia and is the second leading cause of cancer death worldwide causing about a million deaths annually (Kim *et al*., 2010; Yao *et al*., 2011).

Current treatments for this pathology include radiation therapy, surgery and chemotherapy, the latter mainly used in the case of metastases. However, normal tissue damage due to high doses and invasiveness are important limitations for the current treatments required to eradicate cancer cells. In this sense, the use of nanoparticles is a promising area of research in the field of controlled drug release, because these systems allow a wide variety of molecules to be allocated to different tissues gradually over time. Furthermore, the use of nanoparticles minimizes active principle degradation, decreases toxicity, and increases active principle half-life in the organ to be treated (Jain, 2005; Singh, 2005).

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In the last three decades, there have been remarkable advances in the field of nanotechnology, mainly in the use of synthetic polymers. Unlike natural polymers, synthetic polymers have greater biodegradability and biocompatibility (Lee *et al*., 2007). Among the synthetic polymers used in medicine the polylactic acid (PLA) and the co-polymer formed from polylactic acid and glycolic acid (PLGA) are both approved by the FDA (Food and Drug Administration, USA) as drug delivery systems in humans (Zille *et al*., 2010) and other biomedical applications such as making biodegradable sutures (Jain, 2000; Karen & Chuck, 2003). The kind of polymer used to synthesize nanoparticles significantly affects the properties and structure of the particles, and determines its possible applications, even including the way in which it is administered (Pinto Reis *et al*., 2006).

Nanoparticle size appears to be an important aspect. According to Schädlich *et al*. (2011) nanoparticles between 111 and 141 nm in diameter are efficiently accumulated in tumor tissue, while the liver quickly removes larger particles. Similarly, Yu *et al*. (2007) indicate that a continuous administration of PLA nanoparticles results in the accumulation of nanoparticles in tumor tissue.

PLA nanoparticles have been used frequently in cancer therapy (Yang *et al*., 2011). PLA can be produced by the esterification of lactic acid (Zhang *et al*., 2005), one of the simplest chiral molecules in the cell cytoplasm. Lactic acid participates in anaerobic respiration (lactic fermentation), and during glycolysis the pyruvate is converted into lactic acid. Transcriptomic analysis of human breast cancer cells shows that the lactic acid molecule induces proliferative activity and metastasis in cancer cells, upregulates gene expression associated with embryonic stem cells, and activates gene signatures that predict a poor clinical outcome (Martinez-Outschoorn *et al*., 2011).

Copper is an essential trace element for all living organisms participating in various biological functions (Evans *et al*., 2012; Tisato *et al*., 2010) such as development, immune function, neovascularization processes, maintenance of neuroendocrine function and iron metabolism (O'Dell & Sunde, 1997). After iron and zinc, copper is the most abundant mineral in human body. The normal requirements in adults range from 1.2 to 1.5 mg/day, but the body can tolerate 8 to 10 mg/day for several months and 3mg or more for longer periods of time. From yeast to humans, copper is taken up by cells through a family of highly conserved proteins called copper transporters (CTR) (Zhou & Gitschier, 1997). In humans there are two main CTR (CTR-1 and CTR-2). CTR-1 is the major functional protein, whereas CTR-2 mRNA is expressed constitutively, and its function has not been fully elucidated; however, it has been proposed that CTR-2 is related to cell absorption of copper (Blair *et al*., 2009). These transporters are downregulated under high extracellular concentrations of copper, removing CTR-1 from the cell surface by endocytosis and self-degradation (Petris *et al*., 2003).

Copper metabolism is highly regulated by several transporters and chaperone proteins (Furukawa *et al*., 2008). After crossing the plasma membrane, the copper ions are transported to secretory pathways, to mitochondria, and cytosol, for making part of copper enzymes by chaperones Cox 17, Atox-1 and CCS, respectively. Cox 17 drives the copper ions to mitochondrial proteins SCO1 and SCO2, which transfer copper to the cytochrome c oxidase of the respiratory chain (Bertinato & L'Abbe, 2004). ATOX1 can deliver copper to the export pumps ATP7A or ATP7B. Alternatively, ATOX1 can function as a copper-dependent transcription factor of important cell function genes such as cyclin D (Muller & Klomp, 2009). Other data indicate that copper homeostasis is CCS-dependent, where the copper is delivered to SOD1 in mammalian cells (Caruano-Yzermans *et al*., 2006).

Tumor cell resistance and metastases have been related to the human copper transporter CTR-1 present in gastric mucosa (Holzer *et al*., 2006) and in the human gastric adenocarcinoma cell line (Chen *et al*., 2007). In chemotherapy the CTR-1 plays a key role in uptake of platinum drugs, e.g. cisplatin, oxaliplatin, and reduced expression of this transporter as causes tumor cell resistance (Chen *et al*., 2007; O'Connor, 2007).

In this investigation, nanoparticles of polylactic acid (Nano-PLA) and copper-loaded nanoparticles (Nano-Cu) were synthesized as copper carriers to the cancer cell target, and the cytotoxicity presented by these PLA nanoparticle systems on MKN-45 gastric cancer cells was evaluated.

MATERIAL AND METHOD

Cell culture. MKN-45 cells from gastric cancer adenocarcinoma were obtained from Cell Bank, RIKEN BioResource Center and cultured at 37°C with 95% humidity and 5% CO_2 in RPMI-1640 medium (Caisson Labs) containing 10% fetal bovine serum, PSA 1X (amphotericin B, streptomycin and penicillin) and pyruvate (1μM).

PLA and $CuSO_4$-Loaded PLA Nanoparticles. Copper-loaded nanoparticles were obtained using the double emulsion technique (w/o/w) described by Avgoustakis *et*

al. (2002). Briefly, 0.4 ml of copper sulfate aqueous solution ($CuSO_4$, 20mg/ml) was emulsified in 2 ml of dichloromethane solution containing 100 mg PLA (Sigma Aldrich), using 10 W probe sonication for 45 s. Subsequently, the w/o emulsion formed was placed into an aqueous solution of sodium dodecyl sulfate (SDS, 12 mM) and sonicated at 18 W for 1 min. The w/o/w emulsion formed was gently stirred at room temperature until the solvent was completely evaporated. The nanoparticles were purified by applying two cycles of centrifugation (43.370 g) and reconstituted in deionized, sterilized and distilled water. Blank nanoparticles were prepared using the same method, just replacing the aqueous solution of copper sulfate with water.

Characterization of nanoparticles. The characterization of nanoparticles was performed by transmission electron microscopy (TEM) with a JEOL/JEM 1200 EX II microscope and by atomic absorption with a GBC 903 spectrophotometer for copper content of nanoparticles.

Evaluation of cytotoxicity YO-PRO-1/IP. The Membrane Permeability/Dead Cell Apoptosis Kit with YO-PRO ® -1 and propidium iodide (PI) for Flow Cytometry (Invitrogen) was used to distinguish cell apoptosis and necrosis. YO-PRO-1 stain passes selectively through the plasma membranes of apoptotic cells and labels them with moderate green fluorescence, and necrotic cells are stained red-fluorescent with PI that can intercalate with the DNA from permeabilized cells. Fluorescence is read by flow cytometry.

For this experiment, MKN-45 cell lines were grown in 60 mm plates with 3 mL of RPMI medium with 10% of FBS, and 1x PSA antibiotic. After 24 hours of treatment, the cultures were washed twice with 1mL of phosphate buffered saline (PBS) and then 1mL of trypsin was added to each plate to allow the release of cells from the matrix. The cells were placed in an Eppendorf tube for centrifugation for 5 minutes at 3000 rpm. Then the supernatant was removed and 1mL of PBS, 1 µL YO-PRO-1, 1 µL propidium iodide (PI) were subsequently added to each tube. Incubation was performed on ice for 30 minutes in darkness to subsequently carry out a new centrifugation at 3000 rpm for 5 minutes to remove the supernatant. And finally 1mL of PBS was added per tube and analyzed using cytometry flow samples.

Cytotoxicity assays were also performed with nanoparticles loaded with copper exposed for three different periods (24, 48 and 96 hrs). For this case, the treatments were lactic acid (0.04 mM), polylactic acid (0.04 mM), Nano-PLA (0.04 mM), Nano-Cu (0.04 mM/1mM) and copper (1 mM). After the corresponding hours of treatment, the same protocol described above was conducted to analyze the samples by flow cytometry.

Cell cycle analysis by PI. MKN-45 cells were grown in 60 mm plates to assess the cell cycle at three different times 24, 48 and 96 hrs and under 5 different treatments (lactic acid 0.04 mM, PLA 0.04 mM, PLA nanoparticles 0.04mM, Nano-Cu (0.04/1mM, respectively determined) and $CuSO_4$ 1.2 mM). After the corresponding treatment time, the cell cycle was evaluated according to the protocol presented below. Cells were trypsinized for 20 minutes, and the cell suspension corresponding to $1x10^6$ cells was collected. Samples were centrifuged for 3 min at 5000 rpm. The supernatant was removed and the cells re-suspended in 250 µL of PBS at room temperature for 10 minutes and 750 µL of ice cold absolute ethanol added at -20°C for 15 minutes. Samples were centrifuged for 3 min at 5000 rpm, the supernatant removed and 500 µL of PBS added at room temperature for 15 min to allow the cells to rehydrate. 1 µL RNase A (10mg/mL) (Sigma) and 1 µL Triton X-100 (Sigma) was added and incubated at 37°C for 30 min, centrifuged for 5 min at 1500 rpm, the supernatant was removed and added 500 µL of fresh PBS and 1 µL of propidium iodide (500x) (Invitrogen), maintained in the dark and incubated at 4°C overnight. It was then analyzed with a FACS Canto II flow cytometer in the presence of dye.

Cell viability assay (MTT Assay). This procedure is based on the metabolic reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) carried by the mitochondrial enzyme succinate-dehydrogenase in a blue colored compound (formazan), allowing the mitochondrial functionality of treated cells to be determined. Furthermore, this method has been widely used to measure cytotoxicity and cell proliferation. The number of living cells is proportional to the amount of formazan produced (Denizot & Lang, 1986; Mosmann, 1983). The MKN-45 was placed in 96-well plates (Orange Scientific) at a density of 5,000 cells/well. The cells were treated with lactic acid (0.04 mM), PLA (0.04 mM), PLA nanoparticles (0.04 mM), Nano-Cu (0.04/1 mM, respectively) and Cu (1.2 mM) and incubated for 24, 48 and 96 hours. After the treatment period, the culture medium was removed and 100mL of MTT (5 mg/mL) reagent was added to each well and incubated at 37°C for 3 h. 100 mL of DMSO was added to stop the reaction. The plate was read on an Infinite® 200 PRO NanoQuant Spectrophotometer (TECAN, USA) to a 540 nm absorbance.

Statistical analysis compared means using the software Statistica with a p-value ≤ 0.05 for determining the existence of significant differences between the different treatments.

RESULTS

Synthesis and Characterization of Nanoparticles. PLA nanoparticles were synthesized and copper-sulfate loaded as describe above. The particle size and distribution of the PLA nanoparticles were characterized by TEM (Fig. 1A and C). The nanoparticles loaded with copper were slightly larger than those not containing the active principle, and both PLA nanoparticles and copper-loaded PLA nanoparticles samples showed the presence of nanoscale particles (Fig. 1B and D). With regard to the particle size distribution of nanoparticles, PLA nanoparticles showed the highest size frequency between 1-50 nm, with a range of sizes reaching 150- 200 nm (Fig. 1A and B). Nano-Cu presented an enrichment of the population between 50-100 nm, with a wide range of sizes, reaching 300-350 nm (Fig. 1C and D). The atomic absorption analysis determined the concentration of 1mM of $CuSO_4$ in PLA-loaded nanoparticles, and their difference to the total mass confirmed the presence of 40 μM of PLA-synthetized nanoparticles.

Anti-proliferative properties of copper-loaded PLA nanoparticles. The anti-proliferative assay is shown in Figure 2. In addition to lactic acid (40 μM), poly-lactic acid (40 μM), PLA nanoparticles (40 μM), PLA copper-loaded nanoparticles with PLA (40 μM) loaded with copper sulfate (1 mM), and copper sulfate alone (1 mM) were incubated with MKN-45 for 24, 48 and 96 hours, and the anti-proliferative effect was determined using the MTT assay. Our data suggest that the concentration of elemental compounds of nanoparticle structure, lactic acid and unpolymerized PLA do not present any significant differences to their controls. However, the assay presented a significant reduction in proliferation with PLA nanoparticles at 96 h, with PLA copper-loaded nanoparticles at 48, 96 hours and with copper 24, 48 and 96 hours (*$p<0.05$). This demonstrates that copper increased the active cytotoxicity of the PLA nanoparticles in MKN-45 cancer cells. The intermediate result between PLA copper-loaded nanoparticles and the effect of $CuSO_4$ alone may be due to a delayed copper release, with similar results in both samples at 96 h.

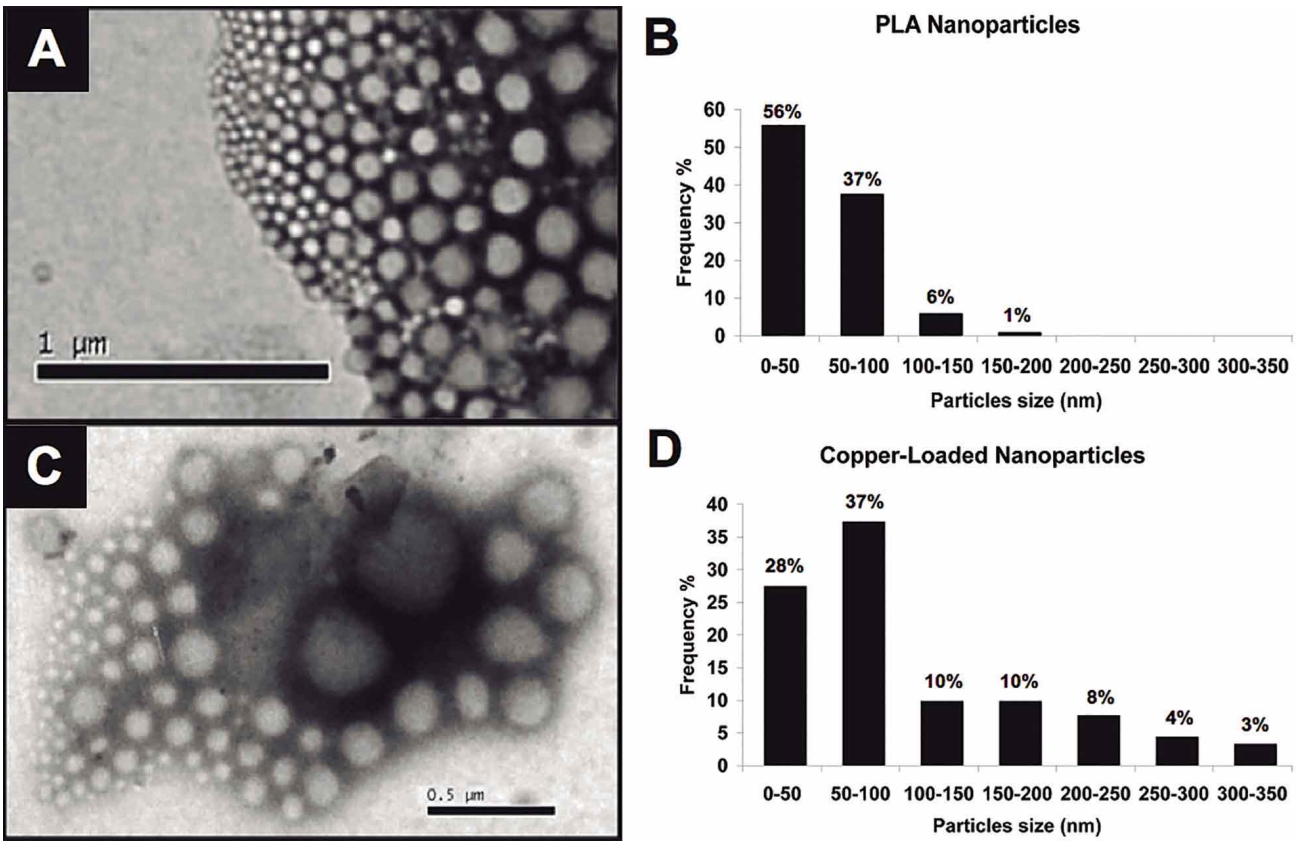


Fig. 1. TEM images of spherical PLA nanoparticle sizes and their distribution (A and B) compared with TEM images of $CuSO_4$-loaded PLA nanoparticles and their distributions (C and D). TEM images of $CuSO_4$-loaded PLA nanoparticles show a larger particle size compared to TEM images of PLA nanoparticles, which show a smaller particle size.

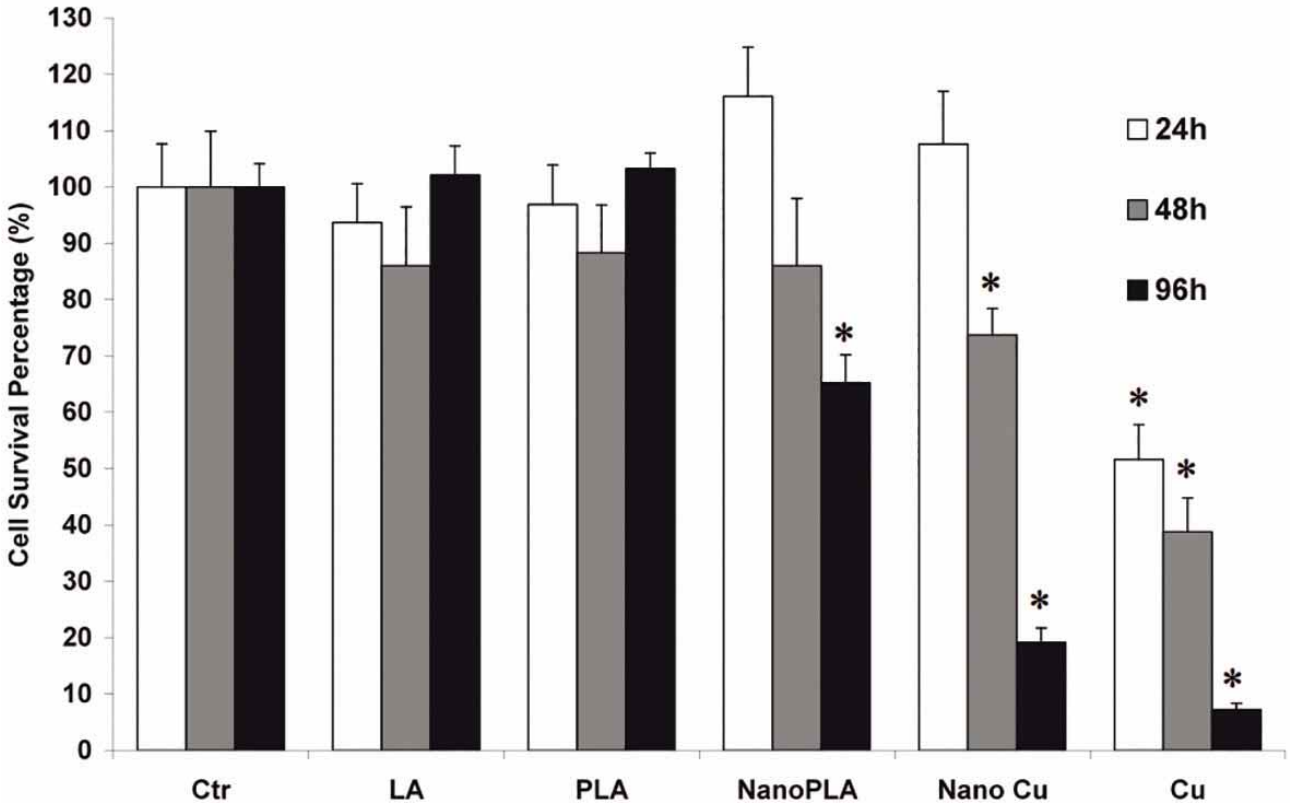


Fig. 2. Effect of lactic acid (LA), poly-lactic acid (PLA), PLA nanoparticles (NanoPLA), $CuSO_4$-loaded nanoparticles and $CuSO_4$ alone on cell survival percentages in MKN-45 cells based on MTT assay. Cells were treated with equivalent quantities of relative compounds 40 μM of LA and PLA and 1mM relative copper for 24, 48 and 96 hours. Yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) was introduced into the 96 wells containing cells incubated for 3 h and measured in a plate reader spectrophotometer TECAN nanoquant. Live cells convert the yellow-colored dye to purple. * indicates a statistical difference from the control, $p<0.05$.

Apoptotic properties of copper-loaded PLA nanoparticles. The MKN-45 cells were analyzed by flow cytometry after treatment with PLA nanoparticles (Nano-PLA) (Fig. 3A–C), and copper-loaded (Nano-Cu) (Fig. 3D–F) for 24, 48 and 96 hours. The cells were harvested for the Vibrant YO-PRO1/IP (Invitrogen) apoptotic assay, which showed early apoptosis in the lower-right quadrant, late apoptosis in the upper-right quadrant and necrosis in the upper-left quadrant. PLA nanoparticles after treatment showed an increase in total apoptosis and necrosis between 24, 48 and 96 hours (Fig. 3A–C). However, copper-loaded PLA nanoparticles presented a higher increase in total apoptosis and necrosis than PLA nanoparticles alone (Fig. 3D–F).

Cell cycle analysis. The anti-proliferative effect of the nanoparticles was corroborated experimentally. Figure 4 illustrates that the treatments with lactic acid (LA), PLA and nano-PLA in unsynchronized cells were unable to induce cell arrest. However, copper-loaded PLA nanoparticles (NanoCu) induced cell arrest in the G1/Go phase (Fig. 4A) with a maximum effect at 96 h, compared to the control, and reduced the S and G2/M phase of the cell cycle (Fig. 4 B and C). This result shows that a specific G1 restriction cell cycle check which have been suggested to be involved in apoptosis induction (Blomen & Boonstra, 2007). The inhibitory effect of copper-loaded PLA nanoparticles on cell proliferation could be explained by the modifications found in the cell cycle analysis.

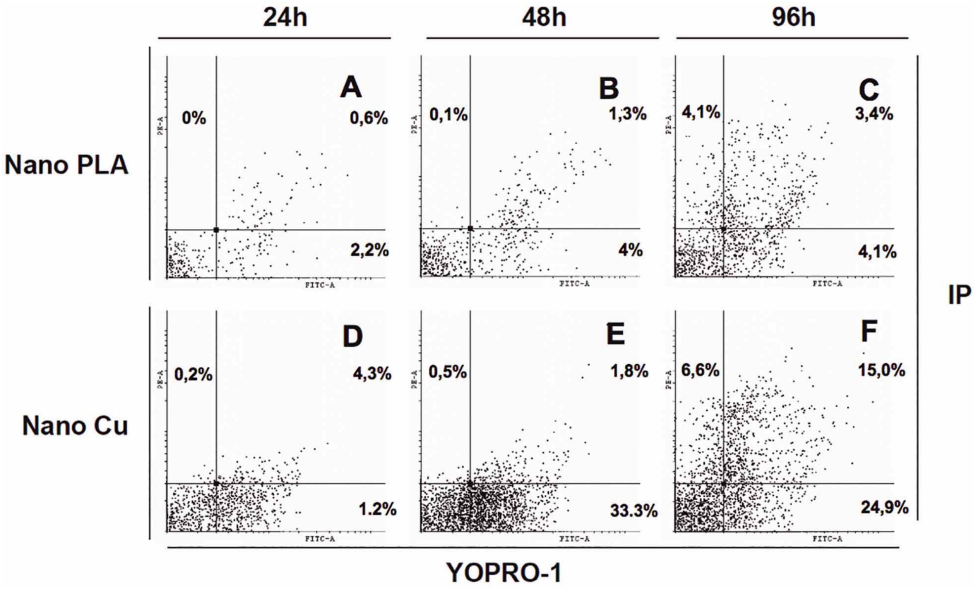
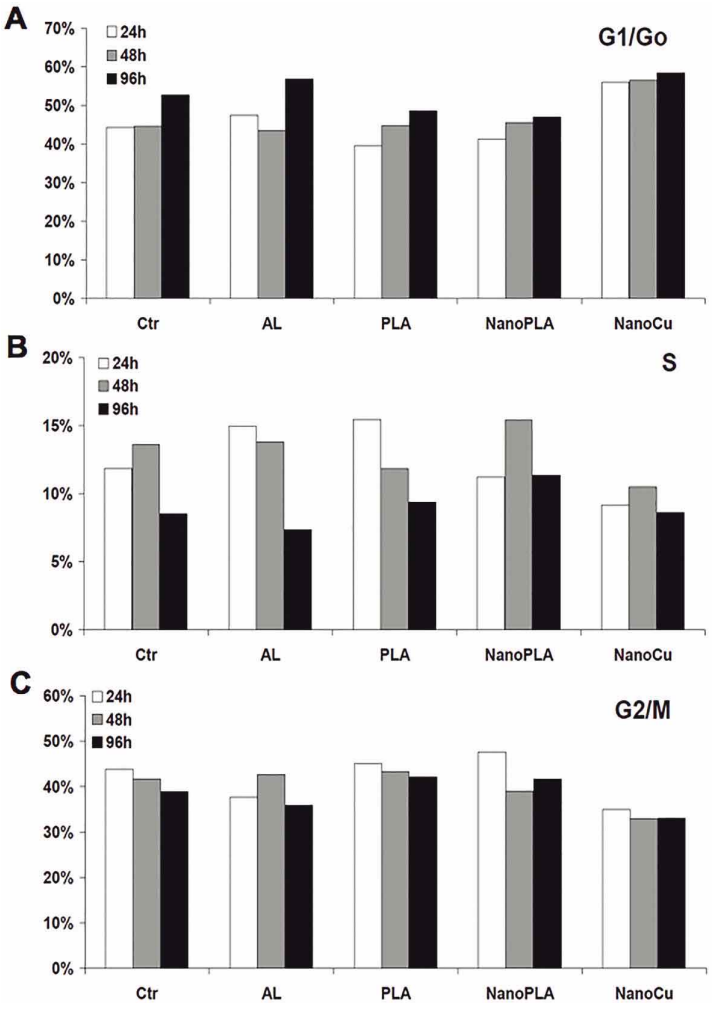


Fig. 3. Flow cytometry analyses of copper-loaded nanoparticle (NanoCu)-induced cell death in a MKN-45 gastric cancer cell line double stained with YO-PRO-1 and PI. MKN-45 cells treated for 24, 48, and 96 hours treatments and each cell quadrant distribution representing viable cells, YO-PRO-1-, PI- (lower left), early apoptosis, YO-PRO-1+, PI- (lower right), late apoptosis/secondary necrosis, YO-PRO-1+, PI+ (upper right) and necrosis, YO-PRO-1-, PI+ (upper left) are shown in each treatment groups. Results shown are one representative experiment.



DISCUSSION

Our study reveals novel data about the in vitro anticancer cell activity of $CuSO_4$-loaded PLA nanoparticles. This finding contributes to the knowledge of drug discovery for cancer treatments.

The causes of tumor cell resistance have been associated with the human copper transporter (CTR-1) present in the gastric mucosa (Holzer *et al.*) and in the human gastric adenocarcinoma cell line related to CTR-1 (Chen *et al.*). CTR-1 plays a key role in the uptake of platinum drugs, e.g. cisplatin and

Fig. 4. Cell cycle distribution of MKN-45 cells 24, 48 and 96 hours after treatments. MKN-45 cells were stained with propidium iodide and the fractions of cells in G1/Go (A), S (B) and G2/M (C) were determined by flow cytometry. Copper-loaded nanoparticles (NanoCu) show a G1/Go increase related to unproliferative differentiated stage, associated with a reduction in mitotic G2/M and S stage.

oxaliplatin, and reduced expression of this transporter causes tumor cell resistance (Chen *et al*.; O'Connor). Certain types of nanoparticles have shown an interesting ability to reverse multidrug resistance, which is a major problem in chemotherapy (Brigger *et al*., 2012). Our results confirm that the double emulsion method described by Avgoustakis *et al*. is efficient in producing nanoparticles under the 150 nm previously declared as optimum for cell tumor accumulation (Yu *et al*.), with an enrichment of 99% for PLA nanoparticles alone and 75% for copper-loaded PLA nanoparticles.

MKN-45 is derived from undifferentiated carcinomas with origin in gastric mucosa (Motoyama *et al*., 1986) and has presented resistance to chemotherapy related to copper transporter (Chen *et al*.). Our results demonstrate that minimal copper sulfate administration (1 mM) is enough to maintain the cytotoxic effect on this cancer cell line with higher significance at longer times (96 h). This result is more related to the copper element than to the sulfate group, as shown in the supplemental data (Fig. 5).

The PLA nanoparticles are derived from polymerization of poly-lactic acid and are approved by the main institutions for drug administration and therapeutics; however, the use of lactic acid polymer is controversial because it has been described as a fuel substrate of cancer cells, in a so-called reverse Warburg effect (Bonuccelli *et al*., 2010). Our results show that there are no significant differences between MKN-45 treated with LA and PLA and its control, and that PLA nanoparticles present a cytotoxic effect on MKN-45 cells, which may be more associated with its nanostructure than with its PLA composition, and when the copper sulfate load is increased, a higher apoptosis effect is produced. The reduced cytotoxic effect of PLA copper sulfate-loaded nanoparticles compared to copper sulfate treatment alone may be explained by the PLA nanostructure causing a delay in the release of content until a similar effect on long-term treatment (96 h) was associated with the degradation of coat PLA produced by the double emulsion method.

The anticancer action on the cell cycle of copper sulfate-loaded PLA nanoparticles is associated with the increase and arrest in the G1/Go state of cell interface, which is associated with the copper sulfate content, more than to the molecular composition of the nanoparticle. Another previous work with PLA nanoparticles showed different patterns of cell cycle arrest is related to the loaded active principle (Xie & Wang, 2005).

All of findings obtained in our results suggest that $CuSO_4$-loaded PLA nanoparticles are effective in inhibiting MKN-45 cancer cell growth, increasing cytotoxicity, apoptosis and G1/Go cell cycle arrest where the anticancer activity was seen with long-term treatment with minimal concentrations of $CuSO_4$ loaded in PLA nanoparticles. Our results indicate that the copper sulfate-loaded PLA nanoparticles are a promising tool for anticancer therapy.

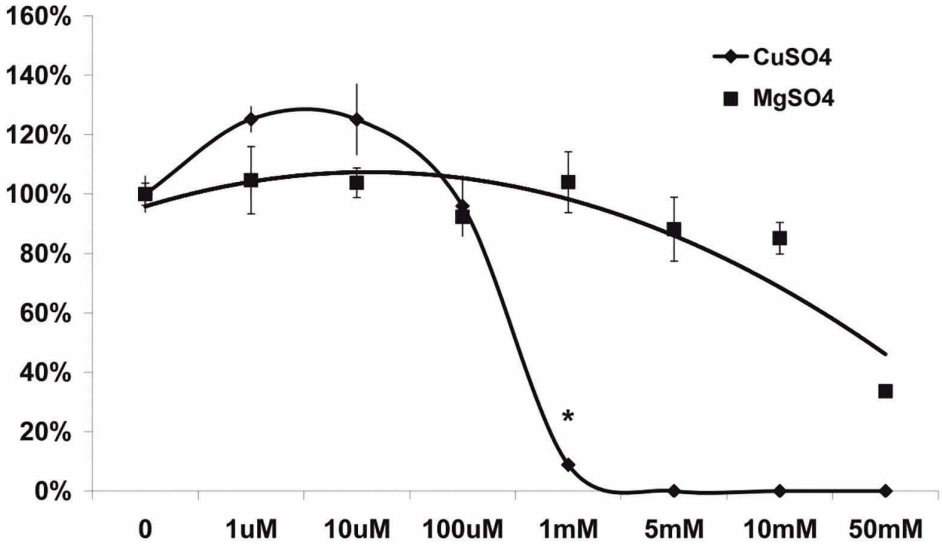


Fig. 5. Effect of $CuSO_4$ and $MgSO_4$ on cell survival percentage in MKN-45 cells based on MTT assay. Cells were treated with equivalent quantities of relative compounds $CuSO_4$ and $MgSO_4$, 0,1 mM, 10 mM, 100 mM, 1 mM, 5 mM, 10 mM and 50 nM, respectively for 96 h. Yellow MTT was introduced into the 96 wells containing cells incubated for 3 h and measured in plate reader spectrophotometer TECAN nanoquant. Live cells convert the yellow-colored dye to purple. *indicate a statistical difference from the control, $p<0.05$.

ACKNOWLEDGMENTS

This research was financial supported by funding from the Universidad de La Frontera through PIA project DI10-7003 and DI10-7006.

MONTIEL-EULEFI, E.; JARA, F.; TORO, C.; GARCÉS, M. & LEAL, P. Efecto citotóxico de nanopartículas de PLA de doble emulsión (w/o/w) cargadas con $CuSO_4$ sobre la línea células de adenocarcinoma gástrico MKN-45. ***Int. J. Morphol., 32(1)***:61-69, 2014.

RESUMEN: Las nanopartículas de ácido poli L-láctico (PLA) tienen la aprobación de las principales instituciones de administración de medicamentos y terapéutica. Sin embargo, el uso de polímero de ácido láctico es controvertido ya que el ácido láctico se ha propuesto como una fuente de energía para las células cancerosas. El objetivo de este estudio fue evaluar las propiedades citotóxicas, la apoptosis y sobre el ciclo celular de las nanoparticulas de PLA biodegradable y de estas PLA nanopartículas cargadas con $CuSO_4$ en la línea celular de adenocarcinoma gástrico MKN-45. Las nanopartículas de PLA para la administración del principio activo $CuSO_4$ contra el cáncer se obtuvieron utilizando el método de doble emulsión. Las nanopartículas de PLA y PLA cargadas con $CuSO_4$ se caracterizan morfológicamente y su tamaño fue determinaron usando microscopía electrónica de transmisión (TEM). Se evaluó la citotoxicidad de este sistema de administración de fármacos utilizando la 3 - (4,5-dimetiltiazol-2-il) -2,5-ensayo difeniltetrazolio (MTT); la apoptosis se evaluó usando yoduro de propidio/YO-PRO-1 y el análisis de ciclo celular por citometría de flujo. Las nanopartículas cargadas con $CuSO_4$-PLA fueron eficaces inhibidores del crecimiento de las células MKN-45 cancerosas. Aumentaron citotoxicidad y la apoptosis, e inducen la detención del ciclo celular en G1/Go, mientras que la actividad contra el cáncer se incrementó con el uso de un tratamiento de 96 horas con una concentración mínima (1 mM) de $CuSO_4$ cargado en nanopartículas con 40 µM de PLA. El tratamiento con 40 µM de ácido láctico y 40 µM PLA no aumentó la tasa de supervivencia de células en relación con el control, lo que indica que el uso de PLA como un polímero portador que no induce la proliferación de células de cáncer MKN-45. Nuestro estudio presenta nuevos datos sobre el efecto de las nanopartículas de PLA con $CuSO_4$ en la línea celular de cáncer gástrico MKN-45.

PALABRAS CLAVE: Nanopartículas cargadas con cobre; Acido poli-láctico; Células de adenocarcinoma MKN-45; Citotoxicidad; Ciclo Celular.

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Received: 18-06-2013
Accepted: 14-11-2013