Biochemical and Biophysical Research Communications 430 (2013) 470–475

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Pulsed extremely low-frequency magnetic fields stimulate microvesicle release from human monocytic leukaemia cells

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ARTICLE INFO

Article history:
Received 29 November 2012
Available online 10 December 2012

Keywords:
Extremely low-frequency magnetic field
Stimulated microvesicle
Calcium homeostasis
Plasma membrane

ABSTRACT

Microvesicles are released from cell surfaces constitutively during early apoptosis or upon activation with various stimuli including sublytic membrane attack complex (MAC). This study shows that an alternating current, pulsed, extremely low-frequency electromagnetic field (0.3 μT at 10 Hz, 6 V AC) induced transient plasma membrane damage that allowed calcium influx. This in turn caused a release of stimulated microvesicles (sMV). When extracellular calcium was chelated with EGTA, sMV biogenesis initiated by ELFMF was markedly reduced and the reduction was less than when the stimulation was the deposition of sublytic MAC. This suggested that pulsed ELFMF resulted in transcellular membrane pores causing organelles to leak additional calcium into the cytoplasm (which EGTA would not chelate) which itself can lead to sMV release.

1. Introduction

Cells typically produce a variety of microvesicles, some of which remain intracellular (for example lysosomes) and others that are released (microvesicles (MVs) or exosomes, collectively known as extracellular vesicles, EV). Microvesicles and exosomes are distinguished according to their mode of biogenesis, size, function and composition [1]. The established characteristics for all MVs, regardless of their cellular origin, include their size, 0.1–1 μm in diameter, and the negatively charged phospholipids such as phosphotidylserine (PS) and phosphotidylcholine (PC) translocated onto the outer leaflet of the plasma membrane as is also seen on MV-producing cells during early apoptosis [2].

Research into the nature of microvesicles has identified various functions [2] including that of intercellular communication, control of apoptosis, cell proliferation and various immunological and procoagulant roles. Furthermore, intracellular pathogens, including certain parasites and viruses, have evolved or adapted to exploit the MV release process to evade host immune responses, invade host cells [3,4] or spread infective particles [5].

MVs are often associated with cellular responses to activation, membrane depolarisation or apoptosis. The processes that govern MV formation and release therefore stem from a cellular response to injury aimed at 'shedding' excesses in Ca_i^{2+} as well as exporting damaging agents [2], thus allowing the cell to recover.

Alternating current (AC)-generated magnetic fields are able to exert electromotive force [6], capable of interacting with polar molecules such as H_2O and more complex organic molecules such as lipid [7]. Alternating currents are used in almost all electrical appliances and power lines generate magnetic fields that extend into the microtesla range, capable of interacting with a range of molecules [7]. Studies on cell lines derived from different tissues and organisms show a whole range of effects [8]. For example, electromagnetic exposure of neuronal progenitor cells resulted in DNA damage and up-regulation of genes relating to apoptosis [9]. These effects were transient and it is conjectured that cellular mechanisms compensate for these magnetic effects. Typically, *in vitro* studies using magnetic fields demonstrate a proliferative stimulus on particular cell lines, initiating G_1 from cells in the G_0 phase [8,10,11].

Calcium influx (in the range 50–300 nM) across plasma membranes as a result of the action of magnetic fields is well documented [7,12]. The subsequent biological effects are often attributed to the excess calcium [13], in as much as calcium is vital for both cytoskeletal remodelling and apoptosis. It has been proposed that F-actin components of microfilaments may interact with magnetic fields, their polyelectrolyte properties allowing calcium ion conductance into the cytosol, analogous to a disjointed conductive cable [11]. This paper proposes that pulsed extremely low-frequency (in the range 3–300 Hz) magnetic fields (ELFMF) induce cell membrane damage, allowing calcium influx, which then initiates the release from cells of stimulated microvesicles.

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http://dx.doi.org/10.1016/j.bbrc.2012.12.012

2. Materials and methods

2.1. Maintenance of cells and determination of viability and cell count

Exponentially growing acute monocytic leukaemic THP-1 cells (AML-M5) obtained from ECACC (Health Protection Agency cell culture collection) (viability ⩾ 95%) were maintained as described previously [14,15]. All sera was rendered MV-free by centrifugation at 20,000*g* for 1 h at 4 °C followed by filtration through a 0.22 μm nitrocellulose filter.

2.2. MV isolation and quantification

This was carried out as described previously [14,15].

2.3. Measurement of intracellular calcium

THP-1 promonocytes were washed and suspended in sterile PBS at a concentration of 1×10^6/ml. Nine microlitres of 50 mM Calcium Green™-1 AM indicator (Invitrogen) (pre-diluted to correct concentration in DMSO, as directed by the manufacturer) was added to the cells and incubated with slow shaking for 45 min at room temperature while wrapped in aluminium foil to minimise photo-bleaching of the fluorophores. The cells were washed 3× in cold, sterile PBS at 160*g* and finally suspended in CGM. Calcium green fluorescence was ascertained on a FLUOstar Ω multiplate reader using the fluorescence programme, stimulating the calcium green at A_{485} and reading calcium green bound fluorescence at A_{520}.

2.4. Stimulation of MV release using ELFMF

A pulsed extremely low-frequency electromagnetic field (ELFMF) was used to provide a low intensity and extremely low-frequency (10 Hz) field. The ELFMF generator plates were housed within the Heraeus incubator (37 °C, 5% CO_2) and the magnetic field was quantified using a TES-1390 EMF Tester on the Tesla option. The AC magnetic field was found to vary from 0.1 to 1 μT depending upon the plate settings, the background field being 0.08 μT while the magnetic field generator was switched on, but falling to negligible levels when the generator was switched off. It was decided to set the plates to generate a 0.3 μT 6 V AC electromagnetic field. The 100 ms duration of the AC field was controlled by a square wave-triggered pulse and the distance between the electrodes to which the THP-1 monocytic cells were exposed was 14 cm.

To assess the use of ELFMFs in generating MVs from cells, THP-1 promonocytes were washed 2× with RPMI 1640 and suspended in CGM. The cells were then assayed for their viability and number using ViaCount reagent (which essentially uses a nuclear and viability dye) on a Guava Millipore flow cytometer before being suspended at the required concentration (typically 5×10^5/ml) and dispensed into 12- or 24-well plates (Corstar). The cells were then stimulated with 0.3 μT at 10 Hz, 6 V AC ELFMFs for 30 min at 37 °C and centrifuged as described in Section 2.2, to isolate sMVs, which were then quantified by flow cytometry using the ExpressPlus programme.

2.5. Flow cytometry assay to identify stages of apoptosis using annexin V-PE and 7-AAD

THP-1 cultured in CGM (>95% viability) were washed 2× by centrifugation at 160*g* for 5 min using RPMI 1640 before being suspended at 5×10^5 cells/ml in CGM. They were dispensed into sterile 12-well plates (Corstar) and the cells were exposed to 0.3 μT 6 V AC ELFMFs within a 37 °C incubator for 30 min or 2 h. After this, the cells were immediately incubated with Guava Nexin® reagent (essentially annexin V-PE, to detect externalised PS and 7-AAD, a cell impermeant dye to detect late-stage apoptosis) following the manufacturer's protocol (Guava, Millipore).

2.6. Incubation of THP-1 promonocytes exposed to ELFMF with propidium iodide

THP-1 cells (5×10^5/ml) were washed 2× with RPMI 1640 and suspended in CGM and dispensed into 24-well plates (Corstar). 5 μM propidium iodide (PI) was added to the cells in culture before one plate was stimulated with 0.3 μT at 10 Hz, 6 V AC ELFMF at 37 °C for 30 min, the control plate being incubated at 37 °C without ELFMF. THP-1 cells were cooled to 4 °C and washed 2× with RPMI 1640 and then suspended in cold PBS before being assessed for PI inclusion by flow cytometry, ExpressPlus and FLUOstar Ω multiplate reader at A_{626}.

2.7. Assessment of lysosomal sealing of ELFMF-induced membrane breach

THP-1 promonocytes (5×10^5/ml) were washed 2× with RPMI 1640 and suspended in CGM before being dispensed into sterile 24-well plates (Corstar). One plate was incubated at 37 °C while the other plate was stimulated with 0.3 μT at 10 Hz, 6 V AC ELFMF at 37 °C for 30 min, after which both plates were rapidly cooled to 4 °C, washed 2× with RPMI 1640 and suspended in sterile PBS. Anti-LAMP-1 Alexa Fluor® (1 μg/10^5 cells) was added to the THP-1 cells and incubated at 4 °C for 45 min, with shaking in the dark. THP-1 cells were then washed 2× with sterile PBS and quantified for LAMP-1 by flow cytometry, using the ExpressPlus software and the FLUOstar Ω multiplate reader at A_{488}. In both cases, a labelled but un-stimulated control was used for gating.

2.8. Calcium chelation during NHS and ELFMF induction of MV release

THP-1 promonocytic cells (5×10^5/ml) were washed 2× with RPMI 1640 and then suspended (in 24-well plates) in RPMI 1640 containing either 10% NHS or being exposed to ELFMF 0.3 μT at 10 Hz, 6 V AC for 30 min at 37 °C with or without 5 mM EGTA and 2 mM $CaCl_2$. The control cells were incubated at 37 °C without exposure to ELFMF. Immediately, the cells were cooled to 4 °C, pelleted and re-suspended in RPMI and their viability assessed by flow cytometry using the ViaCount assay. The supernatant was used for MV isolation and the MV morphology and population count was assessed by flow cytometry using ExpressPlus software (as before).

2.9. Transmission electron microscopy of cells releasing MVs

This was essentially carried out as described before [15], this time MV release from promonocytes (~5×10^6/ml) having been stimulated by exposure to ELFMF (0.3 μT at 10 Hz, 6 V AC) for 30 min.

2.10. Statistical analysis

All experiments were carried out in triplicate and repeated at least three times and the data are represented as mean ± standard error of the mean. Statistical analysis (unpaired *t* test) was performed using GraphPad Prism software, version 5.0 (GraphPad Software, San Diego, CA). The following significance levels were used: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

3. Results

3.1. ELFMF causes small, recoverable decrease in cell viability

ELFMFs disrupt the integrity of the plasma membrane through transient interaction with polar groups associated with phospholipid species. Charged molecules then align with magnetic fields causing regions of low lipid density and forming pores, which allow the flow of extracellular molecules and ions into the cytoplasm along a concentration gradient. We found that application of a 0.3 μT at 10 Hz, 6 V AC magnetic field resulted in an immediate drop in viability from 95% to 89% (Fig. 1A). As soon as the ELFMF was switched off (0 s recovery time), viability was restored to ~94% over the next 3 min and maintained at that level for up to 30 min (Fig. 1B) thus alluding to the removal of damaging agents (by MV release) and restoration of homeostatic mechanisms.

To ascertain the effect of ELFMF density on membrane damage, four field strengths were chosen: 0, 0.1, 0.3 and 0.6 μT at 10 Hz, 6 V AC. We found that with increasing magnetic field density, there was a corresponding significant decrease in viability caused by ELFMF-induced membrane damage (Fig. 1C). A 0.6 μT field density displayed a negligible decrease in cell viability over a 30 min period, compared to 0.3 μT possibly due to the increased field density being able to hinder the diffusion of ions across a rapidly remodelled membrane. Ion channels may also become increasingly distorted, eliminating a possible route of ion transport in denser magnetic fields.

3.2. The effect of ELFMF on levels of apoptosis

The effect of ELFMFs on the release of stimulated MVs (sMVs) from cells without the use of biological or chemical activators was assessed as an alternative method of stimulation. Prolonged (2 h) ELFMF stimulation of THP-1 cells led to a permanent, 18 ± 2%, decrease in total population viability (Fig. 2A). An apoptosis assay (Nexin assay) showed a shunt to early apoptosis (~10%) and late apoptosis (~18%) indicative of pseudoapoptotic events induced by ELFMFs (Fig. 2B and C) which are concomitant with an accumulation of Ca^{2+} in the cytoplasm (Fig. 2D) within 15–30 min of ELFMF exposure.

3.3. ELFMF stimulate microvesicle release from cells

An exposure of THP-1 promonocytes to an ELFMF of 0.3 μT at 10 Hz, 6 V AC (henceforth called ELFMF) for 1 h was found to cause a significant increase (~3-fold) in MV release (1.07×10^5 MVs/ml) compared to control (0.14×10^5 MVs/ml) rested in RPMI 1640 (Fig. 3A and B). This MV release is mediated through the uptake of extracellular calcium as levels of MV release with ELFMF in the presence of EGTA were markedly reduced (Fig. 3A). As shown earlier, this release of stimulated MVs is possibly coupled with pseudoapoptotic events (Fig. 2B). The MVs generated give a typical dot-plot distribution with regard to size and granularity (Fig. 3B) are ~90% annexin V-positive (inset) (indicator of PS expression) as seen by FACs analysis and have a typical morphology and size

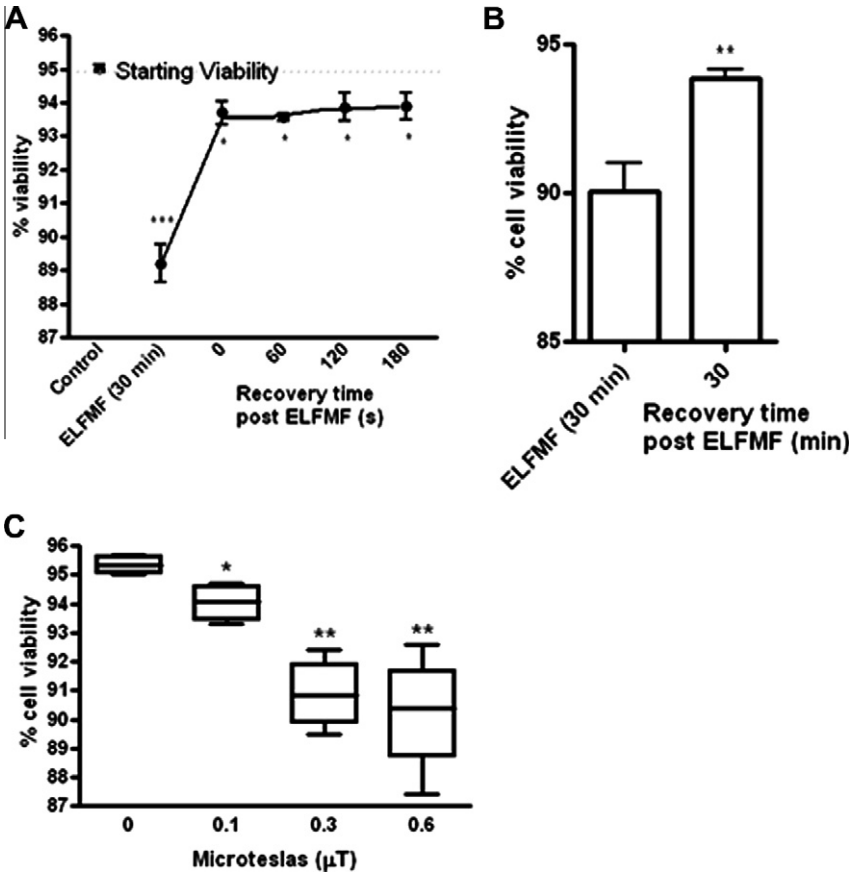

Fig. 1. ELFMFs cause membrane damage facilitating the influx of membrane exclusion dyes and induce pseudoapoptosis for the duration of stimulation. (A) THP-1 promonocytes were stimulated with ELFMFs (0.3 μT at 10 Hz, 6 V AC) for 30 min, temporarily lowering the viability of the cells. Once removed from the ELFMF, THP-1 cells were assessed for their viability every 60 s for 3 min. (B) THP-1 cells were also subjected to 0.6 μT ELFMF strength for 30 min. After the field was switched off, the cells were suspended in complete growth medium and after a 30 min recovery period, there was a significant rise in viability. (C) 1×10^5 THP-1 cells/ml were subjected to ELFMF at three different densities, 0.1 ± 0.05 μT, 0.3 ± 0.05 μT and 0.6 ± 0.05 μT for 30 min and their viabilities assessed using ViaCount.

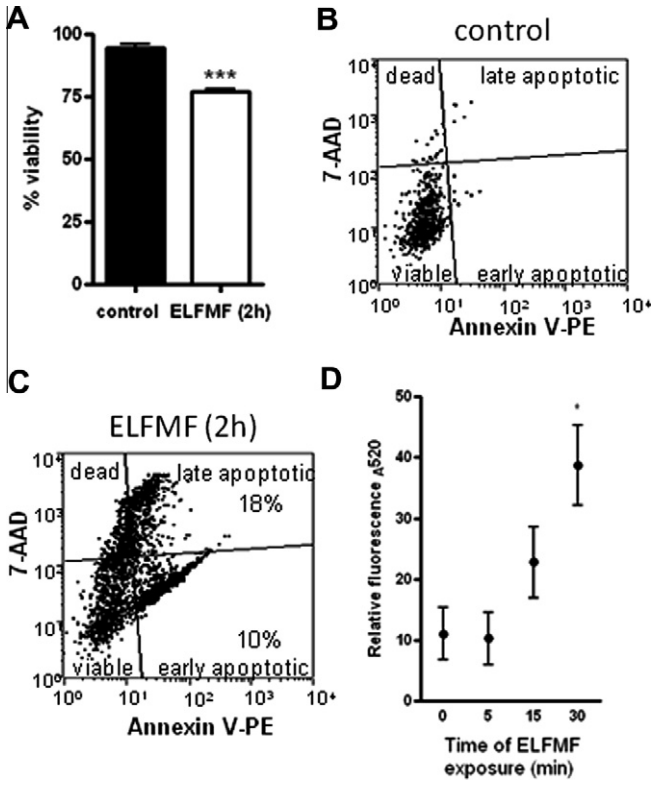

Fig. 2. The effect of prolonged ELFMF exposure on the viability of THP-1 promonocytes. (A) A 2 h stimulation of THP-1 monocytes with ELFMF (0.3 μT at 10 Hz, 6 V AC) resulted in an 18 ± 2% reduction in viability compared to unstimulated control cells. (B and C) An apoptosis assay using AnV-PE and 7-AAD (nexin assay) of THP-1 monocytes stimulated with 0.3 μT at 10 Hz, 6 V AC ELFMF for 2 h showed ~18% of the population to be in late apoptoses. A dot plot obtained on the Guava Easycyte flow cytometer compared ELFMF-treated cells with untreated controls. (D) The cells were quantified for cytoplasmic calcium levels using a FLUOstar Ω multiplate reader exciting at A_{485} and recording at A_{520} at set time points, 0, 5, 15 and 30 min of ELFMF exposure.

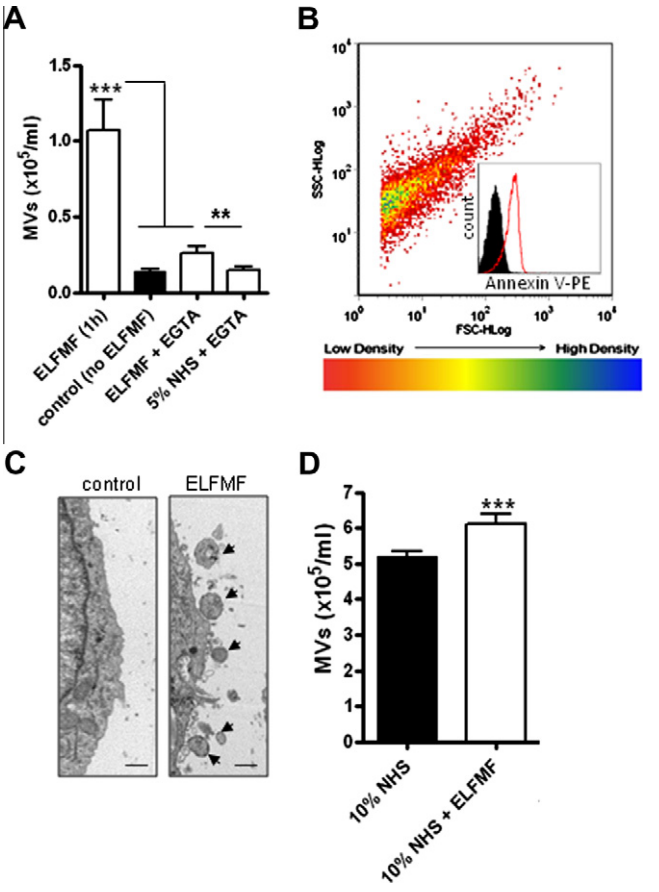

Fig. 3. Identification and quantification of MVs released from ELFMF-stimulated cells. (A) Cell-free culture supernatant from ELFMF-exposed cells was obtained for MV isolation and quantified by FACs analysis (Guava ExpressPlus). There is a significant increase in MV release when cells are stimulated with 0.3 μT at 10 Hz, 6 V AC ELFMFs for 1 h. ELFMF-treated cells in the presence of EGTA (5 mM) showed significantly reduced levels of MV release. However, cells treated with 5% NHS (sublytic MAC) with EGTA, showed an even lower level of microvesiculation, comparable with constitutively released (control) levels. (B) A dot plot morphology obtained by FACs and the annexin V staining of PS, ~90% (inset), confirms MV release during ELFMF stimulation. (C) MV release from THP-1 monocytes was observed by transmission electron microscopy for cells either left uninduced (control) or stimulated with ELFMF for 1 h. Arrows indicate MVs released from the cell surface. The magnification was 10,000× and the scale bar represents 500 nm. (D) MVs are generally released at higher levels when exposed to NHS (10%) and added ELFMF can increase levels of released microvesicles by ~20%.

(200–500 nm in diameter) as seen by electron microscopy of ELFMF-exposed cells releasing MVs (Fig. 3C). They are however produced in lower numbers than MVs generated by other stimuli, such as 10% NHS (Fig. 3D). This suggests that the release of stimulated MVs, as the cells enter early apoptosis, may be an attempt to reduce cellular membrane damage inflicted by the magnetic field. We also found that ELFMFs could add a small but significant level to MV release induced by NHS (Fig. 3D).

As referred to earlier in the introduction, constitutive MV release is in response to normal cellular physiological processes and to cells, having endured various stresses, attempting to prevent the progression of pseudoapoptotic events to irreversible apoptosis. However, prolonged exposure to ELFMFs ensures apoptosis by generating 'un-pluggable' or un-removable membrane pores. Inevitably, cells stimulated with ELFMFs for long enough will initially produce MVs but then progress to apoptosis. As shown earlier, calcium enters cells stimulated with ELFMF for 30 min, significantly increasing total cellular free calcium (Fig. 2D). Calcium influx begins to occur between 5 and 15 min reaching significant levels by 30 min. The lag phase of calcium influx between 0 and 5+ min (Fig. 2D) might occur, as cells are effective at calcium homeostasis and other mechanisms operate to hinder uncontrolled calcium influx. Cells are able to store 'free' calcium within the endoplasmic reticulum (ER); however, stimulation with ELFMFs is likely to cause trans-cellular plasma membrane poration, so that the cellular mechanisms to sequester calcium to the ER become less efficient.

EGTA sequesters Ca^{2+}, so that it is not available to enter the cell. As a result, MV release is markedly reduced. However, microvesiculation levels are reduced significantly below these levels (on a par with background constitutive MV release in the absence of ELFMF or NHS) when EGTA is used to chelate Ca^{2+}-mediated MV release through sublytic pore formation with 5% NHS. This implies that ELFMFs indeed induce trans-cellular poration (as has been suggested before [47]) and that Ca^{2+} from sub-cellular organelles is capable of initiating sMV biogenesis.

3.4. ELFMF stimulates lysosomal exocytosis to repair induced membrane pores

When THP-1 monocytes were stimulated with ELFMF, membrane pores were formed, allowing the influx of membrane impermeant propidium iodide (PI) along a concentration gradient. The PI was retained within the cell after ELFMF stimulation was removed, the relative fluorescence at A_{626} being significantly increased, by 22%, above control (Fig. 4A). As ELFMFs induce the production of

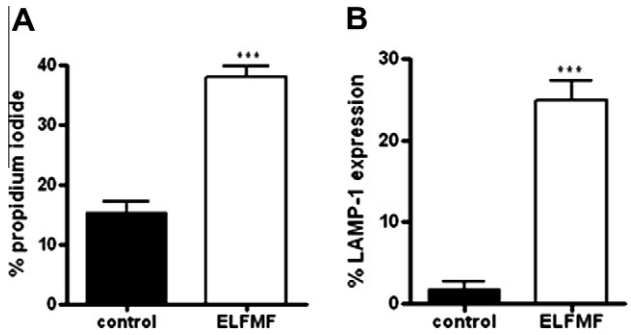

Fig. 4. Exposure of THP-1 cells to ELFMF results in membrane pore formation and LAMP-1 surface expression. (A) Membrane PI levels were quantified on the Guava EasyCyte flow cytometer using ExpressPlus software. Percentage internalised PI was measured in cells both stimulated for 30 min with ELFMF (0.3 μT at 10 Hz, 6 V AC) or not. The stimulated cells showed 22 ± 2% more internalised PI than the control cells. (B) THP-1 cells (5×10^5/ml) were also stimulated with ELFMF and assessed for surface membrane expression of LAMP-1 (CD 107a). Stimulated cells express significantly more Lamp-1, 23 ± 2% than un-stimulated THP-1. LAMP-1 was quantified using a FLUOstar Ω multiplate reader (BMG Labtech).

sMVs through membrane pores, as suggested by internalised PI, so there would appear to be parallels with the mechanism by which sublytic membrane attack complex deposition leads to sMV release [15,16].

The calcium-regulated exocytosis of lysosomes to reseal breaches to the plasma membrane is a recognised mechanism of membrane repair [17]. We wanted to see whether this mechanism might be implicated following ELFMF-induced membrane damage. We found Lamp-1 (lysosomal associated membrane protein 1; CD107a) expression at the plasma membrane of THP-1 cells stimulated with ELFMFs to be 23 ± 2% greater than control (Fig. 4B). It is believed that by causing the polar groups on phospholipids to align with magnetic fields, ELFMFs create outer membrane pores, which allow the influx of ions, causing the cell to enter apoptosis and to release MVs (Fig. 3A and B). Increased cytosolic-free Ca^{2+} leads to increased LAMP-1 at the plasma membrane, by increasing the rate and frequency of lysosomal exocytosis through Ca^{2+}-sensitive secretory pathways [17]. These lysosomes 'plug' the ELFMF-induced pores by fusing with the plasma membrane, preventing the loss of membrane selective permeability and maintaining the membrane's ion gradients.

4. Discussion

Long-term exposure to low magnetic field densities in the 0.1–0.4 μT range may be associated with a host of diseases, amongst others, childhood leukaemia [18], childhood brain tumours [19] and Alzheimer's disease [20]. Microvesicles too as vehicles of intercellular communication have been found to play roles in cancer progression, autoimmune and infectious disease [2]. Extremely low-frequency magnetic fields have an immediate effect on cells [13], and as we found here an ELMF of 0.3 μT at 10 Hz, 6 V AC caused a significant, albeit recoverable decrease in viability for the duration of the 30 min magnetic stimulus. However, prolonged stimulation led to more dramatic decreases in viability, as ELFMFs form irreversible membrane pores (Fig. 2).

Extracellular calcium influx was the main cause for sMV release upon exposure to ELFMFs, as well as the likely release of cellular stores within organelles of Ca^{2+} [21], which contributes to total free cytoplasmic calcium [22]. We found that when extracellular calcium was quenched with EGTA, stimulated MVs (sMVs) were released at a significantly lower level but higher than would be expected through constitutive MV release (no ELFMF) (Fig. 3A). Subcellular organelle pores are stimulated with ELFMF [23], especially in the mitochondria [24], releasing calcium; however, they may release other toxic substances such as peroxide, ROS [13] and Cyt 450 leading to changes in cell viability. sMVs would be expected to export many of these substances from the cell to prevent subsequent apoptosis. However, prolonged ELFMF treatment would ensure their continued supply to the cytoplasm, well beyond the cell's ability to produce and release sMVs, leading to irreversible apoptosis. Notably, Ca^{2+} export by microvesiculation gains importance as a regulatory mechanism especially after ELFMF-induced pore formation in sub-cellular organelles, which prevents effective organelle sequestering of Ca^{2+}.

ELFMF-induced membrane pores were plugged rapidly as observed by immediate lysosomal fusion and the expression of LAMP-1 at the plasma membrane. However, over a 30 min duration, the cells exhaust their supply of lysosomes and membrane pores remain for longer, eventually allowing the increased influx of extracellular constituents such as in this case calcium or impermeant indicator or nucleic acid staining dyes (Fig 2D). When the cells are removed from the ELFMF, PI and calcium, for example, are retained within the cell (Fig. 4A). The increased intracellular calcium, after removal of ELFMF, resulted in the release of sMVs through the usual pathways associated with the onset of early apoptosis. We have established that ELFMFs stimulate sMV biogenesis by causing plasma membrane pores which allow Ca^{2+} influx. Although EGTA chelation of Ca^{2+} led to reduced MV release, ELFMFs stimulate pores throughout the organelles causing free calcium leakage into the cytoplasm, which can thus still stimulate sMV biogenesis and a host of other responses [25].

In this study, we have revealed a new stimulus for MV release from cells. The use of ELFMFs could have important implications in chemotherapy. If ELFMFs and other electroporation technologies cause temporary pores, which can increase effective drug uptake, thus reducing therapeutic levels needed in electrochemotherapy, the observation that ELFMFs also cause the release of microvesicles must be considered as an opposing factor through the potential removal of drug from the cell. Furthermore, the effects of electromagnetic fields, particularly pulsed ELFMF have been described and speculated upon for years, there being much accumulated evidence now for their involvement in cell damage, affecting viability, proliferation and tumorigenesis. The release of sMVs from ELFMF-treated cells is a novel observation which must be further studied especially in view of MVs themselves having been reported to play significant roles in a host of diseases including cancer (tumorigenesis, metastasis and angiogenesis) [2].

Acknowledgments

This work was supported by the Cellular and Molecular Immunology Research Centre and the European Commission – Leonardo da Vinci programme (1-PTE-LEO02-04635). We thank Maria M. McCrossan of the London School of Hygiene and Tropical Medicine for help with the electron microscopy and Ana Costa for critical reading of the manuscript.

References

[1] B. Hugel, M.C. Martinez, C. Kunzelmann, et al., Membrane microparticles: two sides of the coin, Physiology 20 (2004) 22–27.
[2] J.M. Inal, E.A. Ansa-Addo, D. Stratton, et al., Microvesicles in health and disease, Archivum Immunologiae et Therapia Experimentalis 60 (2012) 107–121.
[3] D.G. Meckes Jr., N. Raab-Traub, Microvesicles and viral infection, Journal of Virology 85 (2011) 12844–12854.
[4] I. Cestari, E. Ansa-Addo, P. Deolindo, et al., *Trypanosoma cruzi* immune evasion mediated by host cell-derived microvesicles, Journal of Immunology 188 (2012) 1942–1952.
[5] S.J. Gould, A.M. Booth, J.E.K. Hildreth, The Trojan exosome hypothesis, Proceedings of the National Academy of Sciences of the United States of America 100 (2003) 10592–10597.

[6] N.H. Pham, S. Ohya, M. Tanaka, et al., Electromotive force and huge magnetoresistance in magnetic tunnel junctions, Nature 458 (2009) 489–492.
[7] R.P. Liburdy, T.S. Tenforde, R.L. Margin, Magnetic field-induced drug permeability in liposome vesicles, Radiation Research 108 (1986) 102–111.
[8] A. Lacy-Hulbert, J.C. Metcalfe, R. Hesketh, Biological responses to electromagnetic fields, FASEB Journal 12 (1998) 395–420.
[9] M. Blank, R. Goodman, DNA is a fractal antenna in electromagnetic fields, International Journal of Radiation Biology 87 (2011) 409–415.
[10] C. Ventura, M. Maioli, G. Pintus, et al., Elf-pulsed magnetic fields modulate opiod peptide gene expression in myocardial cells, Cardiovascular Research 45 (2000) 1054–1064.
[11] J. Gartzke, K. Lange, Cellular target of weak magnetic fields: ionic conduction along actin filaments of microvilli, The American Journal of Physiology – Cell Physiology 283 (2002) 1333–1346.
[12] M. Glogauer, J. Ferrier, C.A. McCulloch, Magnetic fields applied to collagen-coated ferric oxide beads induce stretch-activated Ca^{2+} flux in fibroblasts, American Journal of Physiology 269 (1995) 1093–1104.
[13] M. Simkó, M.O. Mattsson, Extremely low frequency electromagnetic fields as effectors of cellular responses *in vitro*: possible immune cell activation, Journal of Cellular Biochemistry 93 (2004) 83–92.
[14] S. Antwi-Baffour, S. Kholia, Y.K.-D. Aryee, et al., Human plasma membrane-derived vesicles inhibit the phagocytosis of apoptotic cells – possible role in SLE, Biochemical and Biophysical Research Communications 396 (2010) 278–283.
[15] E.A. Ansa-Addo, S. Lange, D. Stratton, et al., Human plasma membrane-derived vesicles halt proliferation and induce differentiation of THP-1 actue monocytic leukemia cells, Journal of Immunology 185 (2010) 5236–5246.
[16] B.P. Morgan, A.K. Campbell, The recovery of human polymorphonuclear leucocytes from sublytic complement attack is mediated by changes in intracellular free calcium, Biochemical Journal 231 (1985) 205–208.
[17] A. Reddy, E.V. Caler, N.W. Andrews, Plasma membrane repair is mediated by Ca(2+)-regulated exocytosis of lysosomes, Cell 106 (2001) 157–169.
[18] C. Malagoli, S. Fabbi, S. Teggi, et al., Risk of hematological malignancies associated with magnetic fields exposure from power lines: a case-control study in two municipalities of northern Italy, Environmental Health 9 (2010) 16.
[19] L. Kheifets, A. Ahlbom, C.M. Crespi, et al., A pooled analysis of extremely low-frequency magnetic fields and childhood brain tumors, American Journal of Epidemiology 172 (2010) 752–761.
[20] A.M. García, A. Sisternas, S.P. Hoyos, Occupational exposure to extremely low frequency electric and magnetic fields and Alzheimer disease: a meta-analysis, International Journal of Epidemiology 37 (2008) 329–340.
[21] H. Deng, D. Wang, R. Peng, et al., The electroporation effects of high power pulse microwave and electromagnetic pulse irradiation on the membranes of cardiomyocyte cells and the mechanism therein involved, Sheng Wu Yi Xue Gong Cheng Xue Za Zhi 22 (2005) 672–676.
[22] L. McKeown, N.K. Moss, P. Turner, et al., Platelet-derived growth factor maintains stored calcium through a nonclustering Orai1 mechanism but evokes clustering if the endoplasmic reticulum is stressed by store depletion, Circulation Research 111 (2012) 66–76.
[23] S.Y. Liu, C.L. Chen, T.T. Yang, et al., Albumin prevents reactive oxygen species-induced mitochondrial damage, autophagy, and apoptosis during serum starvation, Apoptosis 17 (2012) 1156–1169.
[24] B.M. Tenorio, G.C. Jimenez, R.N. de Morais, et al., Evaluation of testicular degeneration induced by low-frequency electromagnetic fields, Journal of Applied Toxicology 32 (2012) 210–218.
[25] A. Pilla, R. Fitzsimmons, D. Muehsam, et al., Electromagnetic fields as first messenger in biological signaling: Application to calmodulin-dependent signalling in tissue repair, Biochimica et Biophysica Acta 1810 (2011) 1236–1245.