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Disruption of the Actin Cytoskeleton Induces Fluorescent Glucose Accumulation on the Rat Hepatocytes Clone 9

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Key Words

2-NBDG • Clone 9 hepatocytes • Confocal microcopy • F-actin • Glucose metabolism

Abstract

Background: Glucose transport and metabolism are highly specialized in hepatocytes. Actin cytoskeleton is fundamental to the maintenance of their morphology as well as to ensure their functionality. Here we study the effect of the actin disrupting natural compounds cytochalasin B and latrunculin A on the glucose metabolism of the Clone 9 rat hepatocytes once the glucose molecule is inside them and the effects of two hormones which main function is regulating the glucose metabolism on the actin cytoskeleton of Clone 9 cells. Methods: F-actin was labeled by using Oregon Green 514 ® phalloidin and glucose inside cells was monitored with the fluorescent D-glucose derivative; 2-NBDG. Observations and measurements were carried out by using a confocal microscope. Results: Nor insulin neither glucagon was able to induce any significant effect in the quantity of F-actin present on Clone 9 cells. But insulin triggers a strong reorganization on the pattern of distribution of F-actin. However, the actin cytoskeleton disruption induced by CB and more efficiently by Lat A caused accumulation of 2-NBDG in cells. Conclusions: These results state that disruption of the actin cytoskeleton induces fluorescent glucose accumulation on the rat hepatocytes Clone 9 suggesting that actin disrupting agents cause a blockage in the glycolytic pathway of Clone 9 hepatocytes.

Introduction

Actin is one of the most conserved proteins in eukaryotic cells [1, 2] and its importance can be divided in two groups of activities; structural and functional. Actin cytoskeleton is essential to cytokinesis, cell adhesion, cell movement and migration, cytosolic traffic and maintenance of the cellular shape [3]. Many natural compounds that interact with the actin cytoskeleton have been discovered in the last years. These molecules disturb the cell integrity and functions [4].

Latrunculin A (Lat A) is a macrolide isolated from the marine sponge *Negombata magnifica*. This compound disrupts the actin cytoskeleton by sequestering G-actin (monomeric) and avoiding its polymerization [5-

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7]. Cytochalasins constitutes another group of actin disrupting compounds. These fungal metabolites inhibit actin polymerization by capping the barbed-end of the actin filament [8]. Therefore, both macrolides disrupt actin cytoskeleton by using different action mechanisms. Many other macrolides affects actin cytoskeleton such as halichondramide, swinholide or pectenotoxin [4, 9-13].

On the other hand, D-glucose uptake and metabolism is one of the most studied routes in cells. Any failure in this metabolic pathway frequently entails severe disorders in the organism. Recent investigations discovered that 2-NBDG (2-{N-[7-nitrobenz-2-oxa-1, 3-diazol 4-yl] amino}-2-deoxyglucose), a fluorescent analogue of the D-glucose that gets into cells by using the same transporters [14-18], could be used to monitor the rate of glycogen synthesis in cells [19].

D-glucose uptake by cells and the actin cytoskeleton have been previously related. Actin disruption inhibits D-glucose uptake in most of cells by avoiding GLUT 1 and 4 to reach the plasmatic membrane because they are stored in vesicles inside the cell [20, 21]. However so far there is no information about how drugs that modify cytoskeleton could affect glucose metabolism in hepatocytes.

In this study, we evaluated the effect of compounds that disrupt the actin cytoskeleton on the metabolism of D-glucose. In order to do that, rat hepatocytes from a cell line (Clone 9) were exposed to Lat A or cytochalasin-B (CB) and the consequent changes in the actin cytoskeleton and D-glucose metabolism were evaluated.

Materials and Methods

Latrunculin A (Lat A) derived from *Negombata magnifica* and Cytochalasin-B (CB) from *Drechslera dematioidea* were purchased from Sigma-Aldrich (Madrid, Spain).

The fluorescent dye Oregon Green® 514 Phalloidin for F-actin labeling and 2-NBDG (2-{N-[7-nitrobenz-2-oxa-1, 3-diazol 4-yl]amino}-2-deoxyglucose) were from Molecular Probes (Leiden, The Netherlands).

Cell culture: Nutrient mixture F-12 Ham Kaighn's modification, streptomycin sulphate salt and penicillin G potassium salt were purchased from Sigma (Madrid, Spain) and foetal porcine serum was from Gibco (Barcelona, Spain).

Bovine Serum Albumin (BSA) was purchased from ICN Biomedicals, Inc. (Ohio, U.S.A.).

Drugs: Glucagon was from Novo Nordisk Pharma S.A. (Madrid, Spain) and insulin from Intervet S.A. (Salamanca, Spain).

All other chemicals were reagent grade and purchased from Sigma-Aldrich (Madrid, Spain) or Panreac (Barcelona, Spain).

Buffer solution for confocal assays (in mM): 138 NaCl, 5.5 D-glucose, 5 KCl, 1 $MgCl_2$, 1.5 $CaCl_2$ and 10 Hepes. pH 7.4.

Cell culture

Rat hepatocytes from the cell line Clone 9 (ECACC Nº 88072203) were grown on 60 mm tissue culture plates in F-12 Ham Kaighn's modification supplemented with 2.5 g L^{-1} $NaHCO_3$, 28 mg L^{-1} streptomycin sulphate salt, 17 mg L^{-1} penicillin G potassium salt and 10% fetal bovine serum pH 7.2. The hepatocytes were grown in a humidified atmosphere with 5% CO_2 at 37 ºC [22]

Hepatocytes were seeded on coverslips, which were placed in 8-well sterile plates. Coverslips were used when the cells reached confluence. In this moment cells are ready to one of the following protocols.

F-actin cytoskeleton staining

Once hepatocytes reach confluence, cells were incubated with drugs for 3 h in the culture medium. After that, hepatocytes were washed with PBS and fixed with 4% paraformaldehyde solution (10 min). Then, cells were permeabilized for 5 min with PBS-0.1% Triton X-100 and incubated with 0.165 µM Oregon Green® 514 Phalloidin for F-actin labeling for 20 min. Coverslips were mounted on slides with 1:1 glycerol–PBS and sealed with nail varnish in order to preserve fluorescence and stored at 4 ºC [9, 11]. Control cells were incubated in the same conditions with the toxin vehicle, dimethylsulfoxide (DMSO).

Confocal microscopy for visualizing morphology, actin cytoskeleton distribution and measuring

Confocal imaging was registered with a 60x oil immersion objective of a Nikon Eclipse TE2000-E inverted microscope attached to the C1 laser confocal system (EZC1 V.2.20 software; Nikon Instruments Europe B.V., The Netherlands). A 488nm Argon laser was used to excite orengon green ® 514 phalloidin.

Fluorescent images shown as volume-render projections belong to Z-stacks acquired at 0.5-µm intervals at 512x512 pixel resolution.

Fluorescence measurements corresponding to F-actin labeling were registered and calculated with quantification software from Z-stack sections. Values from all independent experiments were averaged for a single data point.

Results are presented as the percentage of the mean value ± standard error of the mean (s.e.m.) of fluorescence emitted by cells treated with drugs, versus controls, with n = 3.

Fluorescent detection of D-glucose metabolization with 2-NBDG

Hepatocytes were washed 3 times with the buffer solution for confocal assays (cited above in Materials section) and then incubated in buffer solution with 500µM 2-NBDG at 37ºC for 3 h. Then, fluorescent dye was removed by washing 3 times with the buffer solution for confocal assays. Then cells were incubated with drugs or vehicle for 3 h.

Confocal microscopy for visualizing and measuring

Fluorescence measurements and images were registered with a 60x oil immersion objective of a Nikon Eclipse TE2000-E

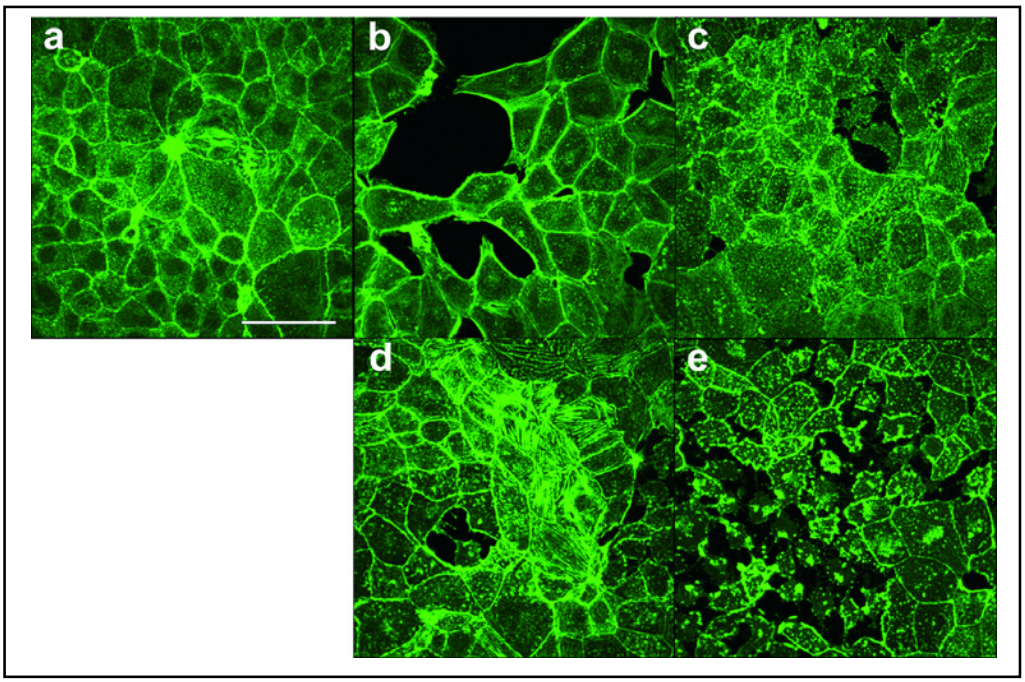

Fig. 1. Images of the effect of actin disrupting agents on the actin cytoskeleton of Clone 9 hepatocytes. Confocal imaging of F-actin labeled with Oregon Green® 514 phalloidin. Control (a) and Clone 9 cells incubated for 3h with 1 or 2 µM CB (b and c respectively) 100 or 200 nM Lat A (d and e respectively). Images are representative from 3 independent experiments. Scale bar = 50 µm.

inverted microscope attached to the C1 laser confocal system (EZC1 V.2.20 software; Nikon Instruments Europe B.V., The Netherlands). Fluorescent images shown as volume-render projections belong to Z-stacks acquired at 0.5-µm intervals and 512x512 pixel resolution.

Fluorescent images are shown as volume-render projections that belong to Z-stacks. Z-stack is a collection of images superimposed taken by Z-scan of the sample at 512x512 pixel resolution. Z-scans are acquisitions taken each 0.5 µm along the Z axis of the sample by confocal software with a motorized focus.

Fluorescence measurements corresponding to 2-NBDG labeling were registered and calculated with quantification software from Z-stack sections. Values from all independent experiments were averaged for a single data point.

Results are presented as the percentage of the mean value ± standard error of the mean (s.e.m.) of fluorescence emitted by cells treated with drugs, versus controls, with n = 3.

Statistical analysis

Results were analyzed using the Students´s t-test for paired data where appropriate. A probability level of =0.05 was set to indicate statistical significance.

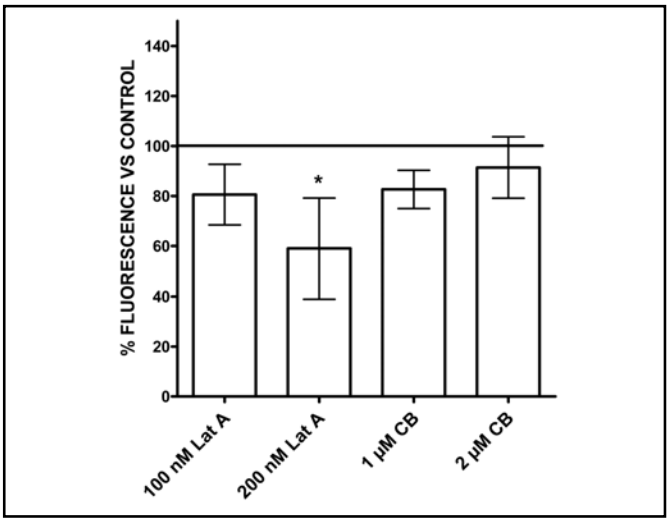

Fig. 2. F-actin fluorescence measurements of Clone 9 hepatocytes treated with actin disrupting agents. The present diagram represents the percentage (mean±s.e.m for n=3 experiments) of fluorescently labeled F-actin in cells treated for 3h with 1 or 2 µM CB either 100 or 200 nM Lat A relative to control cells (100%). Mean±s.e.m of n=3 experiments. *P<0.05; *P<0.01, significantly different from control; Student's t-test.

Results

Clone 9 rat hepatocytes were incubated for 3 h with 1 or 2 µM CB, 100 or 200 nM Lat A or vehicle (dimethylsulfoxide) and then stained for F-actin labeling with Oregon Green ®-phalloidin as described in Materials and Methods section. 1 µM Cytochalasin B induced slight changes in the pattern of distribution of F-actin when compared to control cells (Fig. 1B and 1A respectively). Control cells showed an F-actin meshwork distributed all along the cell body with intensely fluorescent bundles in the cell cortex corresponding to the stress fibers (Fig. 1a). F-actin labeling of 1 µM CB treated cells seemed less intense and some punctuated fluorescence appeared (Fig.1b). This effect was more pronounced in cells treated

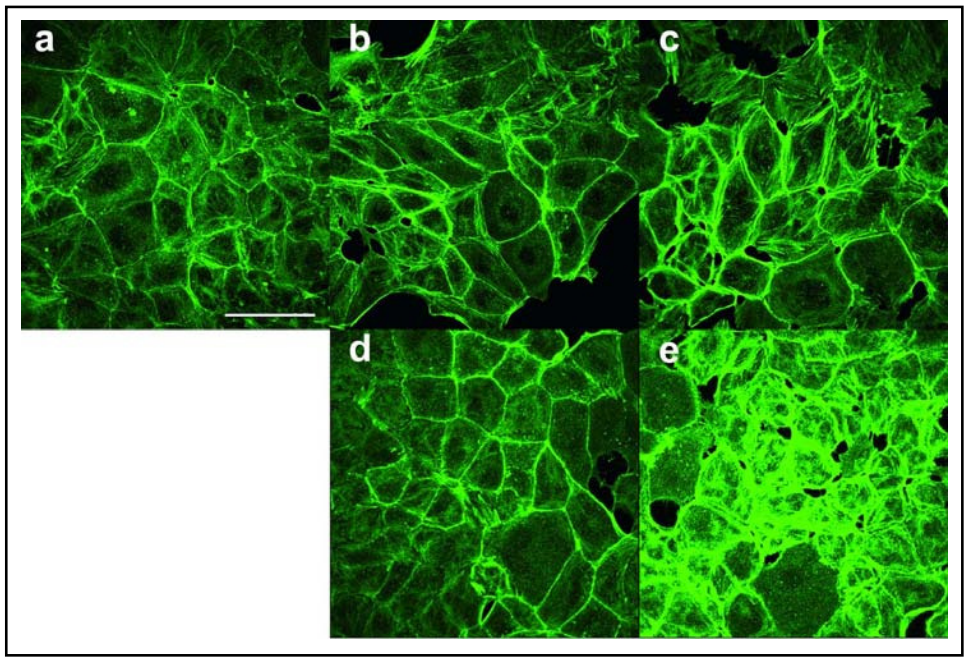

Fig. 3. Effect of agents that alter the glucose metabolism on the actin cytoskeleton of Clone 9 hepatocytes. Confocal imaging of F-actin labeled with Oregon Green® 514 phalloidin. Control Clone 9 cells (a) and Clone 9 cells incubated for 3h with 10 nM or 1 μM glucagon (b and c respectively) 100 nM or 1 μM insulin (d and e respectively). Images are representative from 3 independent experiments. Scale bar = 50 μm.

with 2 μM CB (Fig. 1c) but decrease in fluorescence value was no evident (Fig. 2). 100 nM Lat A was enough to change actin cytoskeleton on Clone 9 cells in a similar way. F-actin decreased and became punctuated (Fig. 1d). 200 nM Lat A disrupted F-actin acutely. Fluorescence disappeared in many parts of cells and F-actin meshwork and bundles was almost replaced by fluorescent dots (Fig. 1e). Fluorescence quantification revealed a significant decrease in the F-actin signal when cells were treated with Lat A, being more pronounced with the higher concentration of toxin (Fig. 2).

Then, Clone 9 cells F-actin distribution was studied after 3h incubation with 10 nM or 1 μM glucagon either 100nM or 1 μM insulin. We used these two hormones that modify in different ways the glucose metabolism to test their effect on the actin cytoskeleton of Clone 9 cells; insulin enhances the glycogen formation and the glucose uptake and glucagon decreases the glycogen formation and increases the glucose release in hepatocytes. Glucagon was not able to exert any significant effect on the F-actin pattern of distribution or in the quantity of F-actin in Clone 9 cells (Fig. 3b, 3c and 4). However, insulin seemed to induce a localization change on fluorescence since the cortical bundles that usually appear in the basal zone of the cells (the adherent zone) showed more intense (Fig. 3d and 3e). But this appreciation was neither reflected in the total volume of the cells nor on the fluorescence values (Fig. 4).

Finally, experiments were carried out to evaluate the effect of Lat A and CB on the D-glucose metabolism of Clone 9 cells. Cells were preincubated with 500 μM 2-NBDG for 3h. Then, cells were washed and incubation buffer was replaced by new solution without 2-NBDG. At this point, drugs were added and after 3h of incubation fluorescence values and images were registered. Cells incubated with 1 μM CB maintained their shape and the fluorescence seemed to be unaltered (Fig. 5b). However, 2 μM CB induced morphological changes in some cells

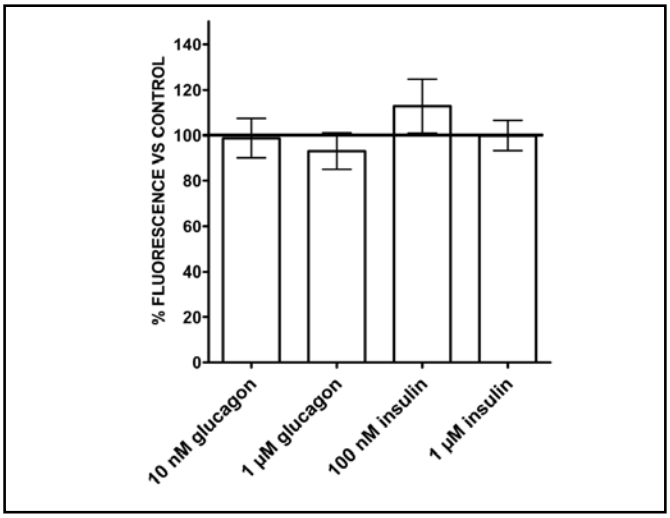

Fig. 4. F-actin fluorescence measurements of Clone 9 hepatocytes treated with agents that alter the glucose metabolism**.** The present diagram represents the percentage (mean±s.e.m for n=3 experiments) of fluorescently labeled F-actin in cells treated for 3h with 10 nM or 1 μM glucagon either 100 nM or 1 μM insulin relative to control cells (100%). Mean±s.e.m of n=3 experiments.

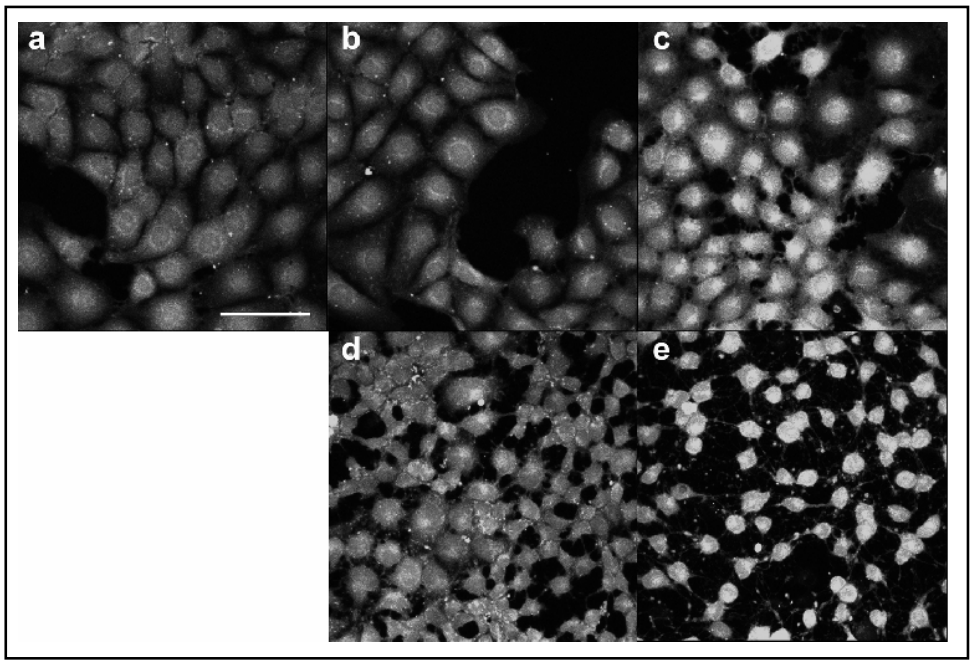

Fig. 5. Effect of actin disrupting agents on the glucose metabolism of Clone 9 hepatocytes. 2-NBDG was included in the incubation media of Clone 9 cells and then released. After, cells were exposed for 3h to 1 or 2 μM CB (b and c respectively), 100 or 200 nM Lat A (d and e respectively) either drugs vehicle (a; control cells) for 3h. Images are representative from 3 independent experiments. Scale bar = 50 μm.

appearing rounder than the control cells (Fig. 5c and 5a respectively). Moreover, the fluorescence intensity seemed increased as it could be seen in Fig. 6. However Lat A is the drug which has drastic significant effects. This compound triggered a sharp change in the morphology of the Clone 9 cells; cell bodies were retracted and rounded loosing their leaf-like appearance (Fig. 5d and 5e). Also, fluorescence clearly increased when cells were treated with 100 nM Lat A, and even more acutely when treated with 200 nM (Fig. 6).

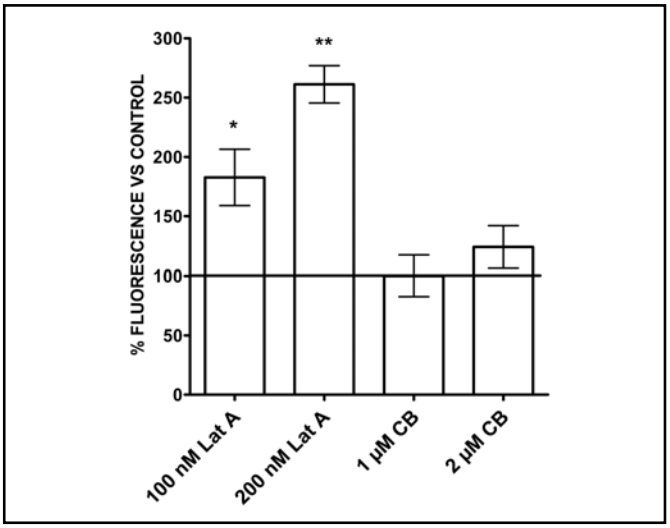

Fig. 6. 2-NBDG fluorescence measurements of Clone 9 hepatocytes treated with actin disrupting agents**.** The present diagram represents the percentage (mean±s.e.m for n=3 experiments) of 2-NBDG in cells treated for 3h with 1 or 2 μM CB either 100 or 200 nM Lat A relative to control cells (100%). Clone 9 cells were preloaded with 2-NBDG by exposing them to the glucose indicator for 3h. Mean±s.e.m of n=3 experiments. *P<0.05; *P<0.01, significantly different from control; Student's t-test.

Discussion

A broad interaction between the actin cytoskeleton and glucose has been previously demonstrated. Uptake of D-glucose is mediated by the protein families GLUT and SGLT [23, 24]. GLUT 1 and GLUT 4 are stored in cytoplasmatic vesicles that are transported to the plasma membrane. Insulin is a hormone that enhances the glucose uptake by cells and the glycogen deposition mainly in hepatocytes. This compound was demonstrated to induce the translocation of the GLUT vesicles from the cytosol to the plasma membrane by a mechanism that implies a strong reorganization of the F-actin framework [25-27]. In this study, insulin did not produce significant changes in the quantity of F-actin. But, the appearance of very intensely labeled F-actin bundles (in the nearest plane to the substrate) shows that insulin induces evident changes in the pattern of distribution of F-actin on Clone 9 hepatocytes. However, glucagon, hormone that enhances the glycolysis and inhibits the glycogen formation, did not change the pattern of distribution or quantity of the F-actin of Clone 9 cells.

Glycolysis is controlled by several mechanisms such as allosteric regulators as well as by reversible binding of glycolytic enzymes to the cytoskeleton. In fact, all the

glycolytic enzymes are described to bind to cytoskeleton with the exception of hexokinase that reversible binds to mitochondria [28]. This mechanism has attracted much attention and has been identified as an important mechanism to generate ATP in the vicinity of the cytoskeleton modulating cellular functions and morphology. In our study, we reveal the effect of diverse actin disrupting agents in the glucose metabolism of Clone 9 hepatocytes by using 2-NBDG, a fluorescent D-glucose derivative that accumulates in living cells and not in dead cells. This dye was used in experiments of cell viability and glucose transport in different cellular models [14, 16, 29-34]. 2-NBDG is not cell permeable and enters in alive cells through the same transporters than D-glucose [14, 15, 35], then it is transformed into a still fluorescent 2-NBDG metabolite [16, 17]. Once this dye is inside the cells, it can follow the D-glucose degradation route or the glycogen formation pathway. In a previous work, we set up a method by using 2-NBDG that allows a noninvasive detection of glycogen being also a rapid testing of drugs that modify the incorporation or release of glucose from glycogen [19].

Cytochalasins are a family of fungal metabolites widely used to study the role of actin in cellular processes and to probe basic aspects of actin polymerization *in vitro*. In Clone 9 cells, CB enables 2-NBDG accumulation. However, CB and CD are potent inhibitors of glycogen synthesis in hepatocytes by an indirect mechanism that probably involves the actin disruption effect [36]. In fact, Baque et al. showed that disruption of actin cytoskeleton by CD enables a dramatic change in the cellular distribution of glycogenin, enzyme implicated in the synthesis of new glycogen particles *de novo* [37]. Therefore, CB may be avoiding normal degradation of 2-NBDG in hepatocytes by a direct or indirect pathway.

It has been reported that glycolytic enzymes are aggregated into multienzyme complexes and that these complexes may confer kinetic advantages, allowing for rapid reaction times and channeling of substrates. The binding of glycolytic enzymes to cytoskeletal structures may be important in this respect and may also facilitate the translocation of enzymes to different parts of the cell in response to changes in the metabolic demands. The glycolytic enzyme aldolase has recently been shown to be associated with the actin cytoskeleton in a reversible and dynamic process which is modulated by glycolytic inhibitors and also by CD [38]. However, CB has a number of effects not related to the F-actin inhibition of polymerization by capping. These include an F-actin severing effect, presumably leading to an increased proportion of shorter actin filaments [39, 40], as well as an inhibition of cellular water permeability [41, 42], possibly via inhibition of the glucose transporter [43]. Furthermore, cytochalasin effects on F-actin in intact cells are highly variable, depending on the cell type in question, as well as on the cytochalasin concentration and congener used. Thus, a given cytochalasin treatment may reduce F-actin content in one cell type, but not in others [44] or differentially affect different F-actin domains within a given cell type [45, 46].Those different CB effects make difficult to directly correlate the actin cytoskeleton disturbance found in hepatocytes with the glycolysis blockage. So, we used another kind of actin disrupting agent which specifically damage cells by disrupting their actin cytoskeleton; Lat A.

Lat A is a macrolide produced by the sponge from the Red Sea *Negomabata magnifica* [5] and a well known G-actin sequestering compound that binds to G-actin with a 1:1 stoichiometry [7]. This compound is a good tool to compare F-actin disrupting agents because its union to monomeric actin is very specific and it does not exert severing or capping activity [4, 6]. Due to its specific activity and significant results we found we can directly relate the effect of Lat A on the actin cytoskeleton of Clone 9 cells with the accumulation of 2-NBDG after its uptake and the glycolysis blockage.

According to research work of recent years, cytoskeleton plays an important physiology role mainly in the following three aspects: insulin secretion, insulin action and the intracellular distribution of glucose metabolism enzymes. In our study, when we use Lat A, we are showing the effect of the actin cytoskeleton disrupting in the glucose metabolism. Many previous and very recent studies have closely related the actin cytoskeleton with the glucose uptake by cells by using Lat A to avoid the actin cytoskeleton correct functioning [47-49]. However, there are not studies that directly correlate the glucose metabolism inside cells with the effect of a specific actin disrupting agent such Lat A. We load the cells with fluorescent glucose (2-NBDG) and observe its evolution with the treatment of cells with Lat A.

The 2-NBDG accumulation that we observed when Clone 9 cells were treated with Lat A could be the consequence of the glycogen formation or a glucose degradation blockage. Glycogen formation with 2-NBDG is characterized by the appearance of a dotted fluorescence pattern, while the disperse fluorescence disappears [19]. In cells treated with Lat A, fluorescence remains accumulated in cells but in a disperse pattern in the whole cell pointing to the glycolytic pathway blockage.

Moreover, it was previously demonstrated that the disassembly of the microfilament-microtubule system leads to a reduction in glycogen synthesis by inhibiting the translocation of glycogen synthase [36, 50].

In summary, we demonstrated that the disruption of the rat hepatocytes actin cytoskeleton, a cell model highly implicated in the glucose regulation in whole organisms, enables the glucose accumulation in cells by using, for the first time, a highly specific anti-actin drug, Lat A.

In conclusion, the impairment on glycolysis that the actin cytoskeleton disrupting agents tested induce in Clone 9 cells seems to be the consequence of a breaking-off in the connection between glycolytic enzymes and their substrates. This impairment of the glucose metabolism could lead to a serious failure in the normal activity of the cell, even to dead.

Abbreviations

2-NBDG (2-{N-[7-nitrobenz-2-oxa-1, 3-diazol 4-yl] amino}-2-deoxyglucose); CB (cytochalasin B); CD (cytochalasin D); F-actin (filamentous actin); Lat A (latrunculin A).

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References

1 Diez B, Marcos AT, Rodriguez M, de la Fuente JL, Barredo JL: Structural and phylogenetic analysis of the g-actin encoding gene from the penicillin-producing fungus Penicillium chrysogenum. Curr Microbiol 2001;42:117-121.

2 Hightower RC, Meagher RB: The molecular evolution of actin. Genetics 1986;114:315-332.

3 Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P: Chapter 16: The cytoskeleton; in S. AMG (ed): Molecular biology of the cell. Garland Science (Taylor & Francis group), New York, 2008.

4 Spector I, Braet F, Shochet NR, Bubb MR: New anti-actin drugs in the study of the organization and function of the actin cytoskeleton. Microsc Res Tech 1999;47:18-37.

5 Kashman Y, Groweiss A, Shmueli U: Latrunculin, a new 2-thiazolidinone macrolide from the marine sponge Latrunculia magnifica. Tetrahedron Lett 1980;21:3629-3632.

6 Spector I, Shochet NR, Kashman Y, Groweiss A: Latrunculins: novel marine toxins that disrupt microfilament organization in cultured cells. Science 1983;219:493-495.

7 Yarmola EG, Somasundaram T, Boring TA, Spector I, Bubb MR: Actin-latrunculin A structure and function. Differential modulation of actin-binding protein function by latrunculin A. J Biol Chem 2000;275:28120-28127.

8 Wessells NK, Spooner BS, Ash JF, Bradley MO, Luduena MA, Taylor EL, Wrenn JT, Yamaa K: Microfilaments in cellular and developmental processes. Science 1971;171:135-143.

9 Ares IR, Louzao MC, Espina B, Vieytes MR, Miles CO, Yasumoto T, Botana LM: Lactone ring of pectenotoxins: a key factor for their activity on cytoskeletal dynamics. Cell Physiol Biochem 2007;19:283-292.

10 Ares IR, Louzao MC, Vieytes MR, Yasumoto T, Botana LM: Actin cytoskeleton of rabbit intestinal cells is a target for potent marine phycotoxins. J Exp Biol 2005;208:4345-4354.

11 Espina B, Louzao MC, Ares IR, Cagide E, Vieytes MR, Vega FV, Rubiolo JA, Miles CO, Suzuki T, Yasumoto T, Botana LM: Cytoskeletal toxicity of pectenotoxins in hepatic cells. Br J Pharmacol 2008;155:934-944.

12 Espina B, Rubiolo JA: Marine toxins and the cytoskeleton: pectenotoxins, unusual macrolides that disrupt actin. FEBS J 2008;275:6082-6088.

13 Saito SY, Watabe S, Ozaki H, Kobayashi M, Suzuki T, Kobayashi H, Fusetani N, Karaki H: Actin-depolymerizing effect of dimeric macrolides, bistheonellide A and swinholide A. J Biochem (Tokyo) 1998;123:571-578.

14 Louzao MC, Vieytes MR, Fontal O, Botana LM: Glucose uptake in enterocytes: a test for molecular targets of okadaic acid. J Recept Signal Transduct Res 2003;23:211-224.

15 Natarajan A, Srienc F: Dynamics of glucose uptake by single Escherichia coli cells. Metab Eng 1999;1:320-333.

16 Yoshioka K, Oh KB, Saito M, Nemoto Y, Matsuoka H: Evaluation of 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose, a new fluorescent derivative of glucose, for viability assessment of yeast Candida albicans. Appl Microbiol Biotechnol 1996;46:400-404.
17 Yoshioka K, Saito M, Oh KB, Nemoto Y, Matsuoka H, Natsume M, Abe H: Intracellular fate of 2-NBDG, a fluorescent probe for glucose uptake activity, in Escherichia coli cells. Biosci Biotechnol Biochem 1996;60:1899-1901.
18 Yoshioka K, Takahashi H, Homma T, Saito M, Oh KB, Nemoto Y, Matsuoka H: A novel fluorescent derivative of glucose applicable to the assessment of glucose uptake activity of Escherichia coli. Biochim Biophys Acta 1996;1289:5-9.
19 Louzao MC, Espina B, Vieytes MR, Vega FV, Rubiolo JA, Baba O, Terashima T, Botana LM: "Fluorescent glycogen" formation with sensibility for in vivo and in vitro detection. Glycoconj J 2008;25:503-510.
20 Wang Q, Bilan PJ, Tsakiridis T, Hinek A, Klip A: Actin filaments participate in the relocalization of phosphatidylinositol3-kinase to glucose transporter-containing compartments and in the stimulation of glucose uptake in 3T3-L1 adipocytes. Biochem J 1998;331:917-928.
21 Wang Q, Khayat Z, Kishi K, Ebina Y, Klip A: GLUT4 translocation by insulin in intact muscle cells: detection by a fast and quantitative assay. FEBS Lett 1998;427:193-197.
22 Espina B, Cagide E, Louzao MC, Fernandez MM, Vieytes MR, Katikou P, Villar A, Jaen D, Maman L, Botana LM: Specific and dynamic detection of palytoxins by in vitro microplate assay with human neuroblastoma cells. Biosci Rep 2009;29:13-23.
23 Bermudez V, Bermudez F, Arraiz N, Leal E, Linares S, Mengual E, Valdelamar L, Rodriguez M, Seyfi H, Amell A, Carrillo M, Silva C, Acosta A, Añez J, Andara C, Angulo V, Martins G: Biología molecular de los transportadores de glucosa: Clasificación, estructura y distribución. Archivos venezolanos de Farmacología y Terapéutica 2007;26:76-86.
24 Wood IS, Trayhurn P: Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. Br J Nutr 2003;89:3-9.
25 Brozinick JT Jr, Berkemeier BA, Elmendorf JS: "Actin"g on GLUT4: membrane & cytoskeletal components of insulin action. Curr Diabetes Rev 2007;3:111-122.
26 Cushman SW, Wardzala LJ: Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. Apparent translocation of intracellular transport systems to the plasma membrane. J Biol Chem 1980;255:4758-4762.
27 Suzuki K, Kono T: Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. Proc Natl Acad Sci USA 1980;77:2542-2545.
28 Meira DD, Marinho-Carvalho MM, Teixeira CA, Veiga VF, Da Poian AT, Holandino C, de Freitas MS, Sola-Penna M: Clotrimazole decreases human breast cancer cells viability through alterations in cytoskeleton-associated glycolytic enzymes. Mol Genet Metab 2005;84:354-362.
29 Leira F, Louzao MC, Vieites JM, Botana LM, Vieytes MR: Fluorescent microplate cell assay to measure uptake and metabolism of glucose in normal human lung fibroblasts. Toxicol In Vitro 2002;16:267-273.
30 Loaiza A, Porras OH, Barros LF: Glutamate triggers rapid glucose transport stimulation in astrocytes as evidenced by real-time confocal microscopy. J Neurosci 2003;23:7337-7342.
31 Louzao MC, Vieytes MR, Botana LM: Effect of okadaic acid on glucose regulation. Mini Rev Med Chem 2005;5:207-215.
32 O'Neil RG, Wu L, Mullani N: Uptake of a Fluorescent Deoxyglucose Analog (2-NBDG) in Tumor Cells. Mol Imaging Biol 2005;7:388-392.
33 Roman Y, Alfonso A, Louzao MC, Vieytes MR, Botana LM: Confocal microscopy study of the different patterns of 2-NBDG uptake in rabbit enterocytes in the apical and basal zone. Pflugers Arch 2001;443:234-239.
34 Zou C, Wang Y, Shen Z: 2-NBDG as a fluorescent indicator for direct glucose uptake measurement. J Biochem Biophys Methods 2005;64:207-215.
35 Gaudreault N, Scriven DR, Laher I, Moore ED: Subcellular characterization of glucose uptake in coronary endothelial cells. Microvasc Res 2008;75:73-82.
36 al-Habori M, Peak M, Agius L: Cytochalasins potently inhibit glycogen synthesis in hepatocyte cultures by an indirect mechanism. Biochem Soc Trans 1991;19:1125-1127.
37 Baque S, Guinovart JJ, Ferrer JC: Glycogenin, the primer of glycogen synthesis, binds to actin. FEBS Lett 1997;417:355-359.
38 Pedley KC, Jones GE, Magnani M, Rist RJ, Naftalin RJ: Direct observation of hexokinase translocation in stimulated macrophages. Biochem J 1993;291:515-522.
39 Cooper JA: Effects of cytochalasin and phalloidin on actin. J Cell Biol 1987;105:1473-1478.
40 Urbanik E, Ware BR: Actin filament capping and cleaving activity of cytochalasins B, D, E, and H. Arch Biochem Biophys 1989;269:181-187.
41 Cornet M, Delpire E, Gilles R: Relations between cell volume control, microfilaments and microtubules networks in T2 and PC12 cultured cells. J Physiol (Paris) 1988;83:43-49.
42 Foskett JK, Spring KR: Involvement of calcium and cytoskeleton in gallbladder epithelial cell volume regulation. Am J Physiol 1985;248:C27-36.
43 Loike JD, Cao L, Kuang K, Vera JC, Silverstein SC, Fischbarg J: Role of facilitative glucose transporters in diffusional water permeability through J774 cells. J Gen Physiol 1993;102:897-906.
44 Downey GP, Grinstein S, Sue AQA, Czaban B, Chan CK: Volume regulation in leukocytes: requirement for an intact cytoskeleton. J Cell Physiol 1995;163:96-104.
45 Cassimeris L, McNeill H, Zigmond SH: Chemoattractant-stimulated poly-morphonuclear leukocytes contain two populations of actin filaments that differ in their spatial distributions and relative stabilities. J Cell Biol 1990;110:1067-1075.
46 Stevenson BR, Begg DA: Concentration-dependent effects of cytochalasin D on tight junctions and actin filaments in MDCK epithelial cells. J Cell Sci 1994;107:367-375.
47 Jewell JL, Luo W, Oh E, Wang Z, Thurmond DC: Filamentous actin regulates insulin exocytosis through direct interaction with Syntaxin 4. J Biol Chem 2008;283:10716-10726.
48 Steinbusch LK, Wijnen W, Schwenk RW, Coumans WA, Hoebers NT, Ouwens DM, Coumans WA, Hoebers NT, Diamant M, Bonen A, Glatz JF, Luiken JJ: Differential regulation of cardiac glucose and fatty acid uptake by endosomal pH and actin filaments. Am J Physiol Cell Physiol 2010;298:C1549-1559.
49 von Lewinski D, Rainer PP, Gasser R, Huber MS, Khafaga M, Wilhelm B, Haas T, Machler H, Rossl U, Pieske B: Glucose-transporter-mediated positive inotropic effects in human myocardium of diabetic and nondiabetic patients. Metabolism 2010;59:1020-1028.
50 Rodriguez-Gil JE, Fernandez-Novell JM, Barbera A, Guinovart JJ: Lithium's effects on rat liver glucose metabolism in vivo. Arch Biochem Biophys 2000;375:377-384.