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Mannose polymer induces vasodilation through a luminal mannose receptor in rat mesenteric arteries

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1. ABSTRACT

Several of the luminal endothelial glycocalyx functions are exerted via interactions with glycosidic components and sugar binding proteins with lectinic activity. One important example is the mannose receptor (MR). The MR has been detected in cell types that mediate the phagocytosis and pinocytosis of particles and solutes containing mannose. Using isolated constant pressurized rat mesenteric arteries (RMA), we evaluated the effects of a mannose polymer in the vascular tone. RMA were pre-contracted with 10μmol/L phenylephrine and carbohydrates were perfused at 20μl/min. Perfusion of free D-mannose [1nmol/L to 100μmol/L] induced a concentration-dependent vasodilation of pre-contracted RMA. Perfusion of mannose polymer [1nmol/L to 100μmol/L] induced a larger effect in a concentration-dependent vasodilation. Mannose polymer's maximum effect reached a 96% of basal diameter; this significant vasodilation was not nitric oxide (NO) or cyclooxygenase (COX) dependent effect. We corroborated the binding of the mannose polymer to the endothelial lumen, by perfusion of a fluorescently labeled mannose polymer; and also, we detected a significant level of MR mRNA in whole mesenteric arteries. With all these, we proposed a novel effect of a MR in the regulation of vascular tone.

2. INTRODUCTION

Vascular endothelial cells are covered with a complex surface layer of membrane-associated proteoglycans, glycosaminoglycans, glycoproteins, glycolipids, and adsorbed plasma proteins (1, 2). Intravital microscopic studies provide evidence for an *in vivo* endothelial surface layer estimated between 0.20 to 0.87μm (1,3,4,5,6,7). Luminal endothelial glycocalyx is directly exposed to the blood stream; it has antithrombogenic properties and modulates leukocyte-endothelial interaction. It also participates in microvascular blood flow resistance (2,8), hence its glycosylated structures may translate hemodynamic stimuli into modulation of parenchymal homeostasis. There is sufficient *in vivo* evidence to suggest that components of the glycocalyx are shed in response to signal transduction events common to inflammation (4,9,10), ischemia-reperfusion (5,6,11), and atherosclerotic processes (12).

Several of the luminal endothelial glycocalyx functions are thought to be exerted via interactions with glycosidic components and sugar binding proteins with lectinic activity (13,14,15). One important example is the mannose receptor (MR) (16). MR is a sugar binding C-type

lectin, type I transmembrane receptor (16,17)), mainly expressed in macrophages (18,19), and liver, spleen and dermal microvascular endothelial cells (18,20,21). MR plays a key role in the homeostasis of these cell types. MR is the prototype for a family of receptors with C-type carbohydrate recognition domains (17). The MR family comprises the Endo180 receptor, the M-type phospholipase A2 receptor (PLA2R), 1 and DEC-205/MR6-gp200. This family grouping is based on an overall structural conservation with the four receptors containing a large extracellular domain comprising an N-terminal signal sequence followed by a cysteine-rich domain, a fibronectin type II domain, and 8 or 10 C-type lectin-like domains (CTLDs)(22,23). A short cytoplasmic domain follows the single pass transmembrane domains. As a family, these receptors have two striking features. First, although they belong to the large C-type lectin superfamily, they uniquely contain multiple CTLDs within a single polypeptide backbone. Second, they share the ability to be recycled between the plasma membrane and intracellular compartments of the cell. The sugar-binding domains are Ca^{2+}-dependent, pH sensitive and have high and selective affinity for terminal mannose residues.

MR has been involved in some of the scavenger functions of macrophages and endothelial cells (16,24). This endocytic process is mediated by focal F-actin polymerization, small Rho-GTPase activation, and p21-activated kinases (25). However, besides this well-know effect of MR, it has also been associated with processes that affect vascular function such as the production of prostaglandins by human macrophages (26).

Previously, it was showed that intracoronary perfusion of mannose polymers (0.1pmol/L to 1nmol/L) increase the positive inotropism induced by coronary flow stimuli in isolated and perfused guinea pig hearts. This inotropic effect was mediated by a lectinic MR-like protein present in the coronary endothelium (27).

In the present work, rat mesenteric arteries were used to analyze the mannose-induced effects, since several physiologically important functions of microvessels are know to be affected by surface characteristics of the endothelium (28). Previous work shows an increase in myocardial contraction that might be related to a vasodilation effect in isolated guinea pig heart. We hypothesized that the MR is present and functional in the endothelium of resistance vessels (< 300μm) and might be involved in vascular responses to flow. Therefore, we evaluated the vasodilatory effects of intraluminal infusion of D-mannose in arteries pre-contracted with phenylephrine; we evaluated the site of D-mannose action by confining D-mannose intraluminarly with a high molecular weight mannose polymer that does not permeate the endothelium. Furthermore, we used RT-PCR to demonstrate the presence of MR mRNA in rat mesenteric arteries. We also explored the participation of cyclooxygenase (COX)-derived autacoids and nitric oxide on the mannose-induced effects of rat mesenteric arteries.

Our results showed that MR is present and active in rat mesenteric arteries and it is involved in endothelium-induced vasodilation.

3. MATERIALS AND METHODS

Experimental protocols were approved by the animal welfare and housing committee of the Escuela Superior de Medicina of the Instituto Politecnico Nacional, Mexico. Reagents unless noted were provided by Sigma-Aldrich (St Luis MO).

3.1. Mesenteric artery isolation

Experiments were performed in male Wistar rats (250-300g) anesthetized with sodium pentobarbital (60mg/kg intraperitoneal). Animals were mechanically ventilated with room air via a tracheal cannula. A median incision of the abdomen was performed to expose a segment of the small intestines and the supporting and feeding mesentery was quickly excised and placed in ice-cold Krebs-Henseleit solution (mmol/L) NaCl 117.8; KCl 6; CaCl2 1.75; MgSO4 1.2; NaH2PO4 1.2; NaHCO3 24.2; glucose 5 and sodium pyruvate 5; solution was equilibrated with 95% O2 5% CO2, pH 7.4. 2 to 3mm segments of the second order branch of the superior mesenteric artery were separated from the surrounding connective and adipose tissues. The mesenteric artery was transferred and cannulated onto glass pipettes in a temperature-controlled tissue chamber, which was carefully mounted onto the stage of the inverted microscope (Nikon Eclipse TS100, Japan) (29,30,31).

Mesenteric arteries were perfused and superfused with Krebs-Henseleit solution, maintained at 37 C and bubbled with 95% O2 and 5 % CO2, throughout the experiment. Each mesenteric artery was stabilized for 5min at a physiological perfusion flow rate (20μl/min) to remove remaining blood, detritus and metabolites. Then, perfusion solution was maintained with no flow for 60 minutes but at a physiological perfusion pressure (60mmHg). Perfusion flow and perfusion pressure was maintained constant with a pressure servo control (LSI) connected to a transducer. Mesenteric artery tone was visualized using an objective 4X (NA0.10; Nikon, Japan) and a CCD camera (Model XC-73, SONY Japan) was used to capture the image. The image was projected on a monitor and digitally recorded. Measurements of the lumen diameter and wall thickness were made with a video dimension analyzer (LSI), as described previously (32).

3.2. Mannose polymer synthesis

Mannose polymer was synthesized from a MW 260,000 dextran matrix, using the method described by Di Virgilio et al (33). Briefly, 1g of Dextran (260,000 Da) was dissolved into 100ml of 0.5mol/L of carbonate sodium solution (pH 9.0). Divinylsulfone (DVS, 10ml) was slowly added drop wise with constant stirring over a 15min period. The reaction was maintained for 1 hour at room temperature (RT). 10mg of D-mannose dissolved in 10ml of 0.5mol/L of carbonate sodium solution pH 9.0 was added to the dextran-DVS activated solution. The reaction was maintained for an additional hour at RT. The addition of 5ml of ethylendiamine blocked excess non-reacted groups and stirring continued for 1hour at RT. To remove unbound D-mannose and ethylendiamine, mannose polymer was extensively dialyzed, using a MW 10,000

cutoff membrane, against distilled water at room temperature. The mannose polymer was precipitated with absolute ethanol, dried and stored upon utilization. To measure the amount of bound mannose in polymer, we measured reducing sugars with 3,5-dinitrosalicylic acid, according to the previously reported method (34,35). Mannose-polymer has 3.5mg of mannose per g of polymer.

3.3 Experimental protocols

The mesenteric artery was set at *in situ* length and allowed to develop spontaneous tone at 60mmHg luminal pressure with no perfusion flow for 1hour. All experiments started once the mesenteric artery developed a constant tone for over a period of 15min. During this period, the perfused pressure is similar to the blood perfusion pressure measured in these arterioles at *in vivo* conditions.

To determine the response to perfusion flow of the mesenteric artery as follows: perfusion flow of the artery was increased stepwise (20µl/min every 5min) from 20 to 100 µl/min at constant perfusion pressure (60mmHg), without previous stimuli by phenylephrine. The artery diameter was measured at each flow step. The vasoconstriction response of the mesenteric arteries was evaluated by addition of phenylephrine in the superfusion solution dissolved in a Krebs-Henseleit solution. The phenylephrine's concentration-response was evaluated from 1nmol/L to 1mmol/L. Each concentration increment was added to freshly washed artery. The perfusion pressure was maintained constant at 60mmHg during the experiment. Mesenteric artery tone was measured at maximum effect developed by each phenylephrine concentration.

The mesenteric arteries were pre-contracted with 10µmol/L phenylephrine, which induced an 80% maximal vasoconstriction to evaluate the flow-dependent vasodilatory effect. 5 minutes later, the perfusion flow was increased from 20 to 100µl/min with step increments of 20µl/min every 5min at a constant perfusion pressure of 60mmHg, as mentioned above measurements were performed at every flow increase.

Other sets of experiments were performed to evaluate the effect of monosaccharides and two different high molecular polysaccharides on the mesenteric arteries. After equilibration at 60mmHg of perfusion pressure for 1hour, the mesenteric artery was pre-contracted with 10µmol/L phenylephrine. 5 minutes of stabilization were arteries incubated, then D-mannose, D-glucose, D-galactose, dextran (260 000 Da), and mannose polymer from 1nmol/L to 100µmol/L were intraluminal perfused. The mesenteric artery diameter was measured at every concentration.

3.4. Mannose receptor mRNA detection and determination of mannose polymer binding site

To evaluate the presence of the mannose receptor on mesenteric arteries, we extracted total RNA and performed RT-PCR to assess MR mRNA of isolated mesenteric arteries. Briefly, after extracting the rat mesenteric arteries, the tissues were snap frozen in liquid nitrogen and kept at –80C. Total RNA was extracted using the Perfect RNATM, Eukaryotic Mini (Eppendorf, Boulder CO). Isolated RNA was quantified using the GENESYSTM 10 series (ThermoSpectronic) and 5µg RNA were placed on a 1.0% agarose gel containing ethidium bromide in MOPS buffer. Running buffer and gel contained 0.2mol/L formaldehyde. To prevent trace amounts of DNA contamination, RNA samples were treated with amplification grade DNase I (Invitrogen, Carlsbad CA) before reverse transcription. All RNA samples were stored at –80C in RNA elution solution until further use.

cDNA Synthesis and real time assays: We used 0.5µg of RNA for reverse transcription with random hexamers in 20µl, using the Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics GmbH, Basel Switzerland) and the reactions were performed in Eppendorf Mastercycle Thermocycler (Eppendorf). The amplified cDNA was quantified at 260nm. RT-PCR reactions were conducted using the Rat Universal Probe Library (Roche Diagnostics GmbH, Basel Switzerland). Specific oligonucleotide primers were originally generated with the online assay design software (Probe Finder; www.universal-probelibrary.com) and the primer sequences of liver MR were forward 5'AGCCACTCCGAACCTGGCAGC3', and reverse 5'TCATGCGACATGGGTTCCTGG3'. 20µl reaction mixture contained 1X LightCycler TaqMan Master reaction mixture (Roche Diagnostics GmbH, Basel Switzerland), 200nmol/L of each primer, 100nmol/L of Universal Probe Library probe, 0.5U LightCycler Uracyl-DNA glycosylase and 2µl of standard DNA in appropriate dilution. The amplification was performed in borosilicate glass capillaries (Roche Diagnostics GmbH, Basel Switzerland).

To analyze whether the mannose-polymer remained confined intraluminal, we indirectly showed that mannose-induced vasodilatory effects were exerted at the endothelial cell level using a FITC-labeled mannose polymer. Mesentery arteries were perfused with labeled mannose polymer by 30 minutes and for another 30 minutes to washout unbound polymer with Krebs-Hensley solution. Rat mesenteric arteries were cut in 1mm slices, mounted with Vecta-shield TM containing propidium iodide and analyzed by epi-fluorescence microscopy.

3.5. Inhibition or blockade of possible mediators of D-mannose-induced vasodilation

To study the possible existence of mediators of D-mannose induced vasodilation in rat mesenteric arteries, several receptor antagonists and enzymatic inhibitors were tested: glibenclamide [1µmol/L] an antagonist of ATP-sensitive K+ (KATP) channels; apamin [10µmol/L] a blocker of Ca2+-dependent K+ (KCa) channels, NG-nitro-L-arginine methyl ester (L-NAME) [10µmol/L], an inhibitor of the nitric oxide synthase; indomethacin [10µmol/L] a COX inhibitor; reactive blue 2 [1µmol/L] a P2Y purinoceptor antagonist; aminophylline [10µmol/L], an adenosine receptors blocker. Each substance tested was continuously perfused during the experiment, 15min after phenylephrine administration and previous D-mannose perfusion

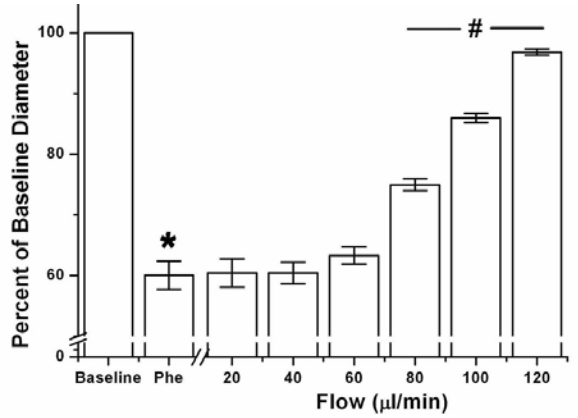

Figure 1. Perfusion Flow induces vasodilation on pre-contracted rat mesenteric arteries. Changes in mesenteric artery diameter were expressed as percent of the baseline diameter. Rat mesenteric arteries were pre-contracted with phenylephrine 10µmol/L (Phe). Step increases of perfusion flow induced statistically different vasodilation after 60µl/min. Data are expressed as mean±SE. * and # compared vs. Baseline diameter, and were statistically different, $p<0.05$.

3.6. Data Analysis and Statistics

Data were normalized against diameter baseline values at constant perfusion pressure and no stimuli. Data represent means ± SE. Each experimental condition had an "n" value of 6 rats. Each rat provided one artery. Other vessels or organs from the same rat were used in other experiments. Comparisons between groups and among treatments were performed using an ANOVA and Bonferoni post-test for individual differences. Differences were considered statistically significant when $p<0.05$.

4. RESULTS

4.1. Mesenteric arteries becomes activated with pressure and flow

The baseline diameter of mesenteric arteries studied at a constant perfusion pressure of 60mmHg was 287±5µm (n=35). Changes in mesenteric artery diameter were expressed as a percentage of baseline diameters. Pressurized mesenteric arteries showed a phenylephrine concentration-dependent vasoconstriction (data not shown). 10µmol/L phenylephrine elicited ~80% of the vasoconstriction obtained with 1mmol/L phenylephrine. In the following experiments, mesenteric arteries were pre-contracted with10µmol/L phenylephrine. In pressurized and non pre-contracted mesenteric arteries, the stepwise increase in perfusion flow did not modify the baseline diameter (data not shown). Further analysis of the perfusion flow effects in pre-contracted mesenteric arteries was realized. The effect of different flow levels (20, 40, 60, 80, 100 and 12 µl/min) effects were tested on rat mesenteric arteries. Figure 1 show that step increase in perfusion flow induces a vasodilatory effect in pre-contracted mesenteric arteries and this effect was statistically significant only at higher perfusion flows than 60µl/min. At perfusion flow to 60µl/min, there was observed a marginal vasodilation. The maximal effect was elicited by a perfusion flow of 120µl/min at 92% of the baseline diameter (fig. 1). There is no significant change in the mesenteric artery diameter at 20 or 40µl/min perfusion flow. Therefore, it was decided to use 20µl/min as the perfusion flow for the following experiments.

4.2. D-mannose and the mannose polymer perfusion increase the vascular diameter

The intraluminal perfusion (at 20µl/min) of D-mannose induced a significant concentration-dependent vasodilatory response (fig. 2), this vasodilation response appeared immediately after D-mannose perfusion started. Mannose perfusion-induced vasodilation was statistically significant at 0.1µmol/L. The maximal vasodilation elicited by D-mannose [100µmol/L] reached 92% of the baseline diameter. These vasodilatory effects are carbohydrate specific, since intraluminal perfusion, at a similar concentration range, of D-galactose and D-glucose induced no changes in the diameter of pre-contracted rat mesentery arteries (fig. 3).

Similar to free D-mannose (fig. 2), the intraluminal perfusion of the mannose polymer produced a vasodilatory effect (fig. 4) in pre-contracted rat mesenteric arteries. It is noteworthy that the mannose-polymer perfusion showed a greater potency. Figure 2 shows that concentration of free D-mannose had to be 100nmol/L to exert significant vasodilation in pre-contracted mesenteric arteries. The mannose polymer effect was present at 100 times lower concentration of mannose than free D-mannose. Maximum effect induced by the mannose polymer was induced by 1µmol/L (96% of the baseline diameter). The same concentration of free mannose only exerted 40% of vasodilation (75% of the baseline diameter) in the pre-contracted mesenteric arteries (figs. 2 and 4).

In another set of experiments, dextran (mannose polymer base matrix) was perfused from 0.01nmol/L to 1µmol/L (2.6µg/L to 0.26g/L to compare with mannose polymer); these concentrations were ~5 times higher than the concentrations of mannose polymer 0.515µg/L to 0.0515g/L (0.01nmol/L to 1µmol/L of mannose content in polymer). Figure 5 shows that dextran 260,000 Da perfusion induced a slightly small concentration-dependent vasodilation. Vasodilatory effect of the dextran perfusion in the pre-contracted mesenteric arteries was only statistically significant at 0.1µmol/L (2.6mg/L) concentration perfused. It is noteworthy that the maximal effect induced by the higher dextran concentrations only elicited a vasodilatory effect that reached 66% of the baseline diameter (fig. 5). Meanwhile, figure 4 shows that the same level of vasodilation (~70% of baseline diameter) was observed when it was perfused 51.5µg/L of the mannose polymer (1nmol/L of mannose content in polymer). These results (50 times-fold) demonstrate that mannose polymer-induced effects are more likely due not by the dextran base matrix itself but by the mannose content of the polymer.

4.3. MR-mRNA is express in mesenteric artery

Figure 6 shows that the rat mesenteric arteries and rat liver express MR mRNA detected by RT-PCR. To

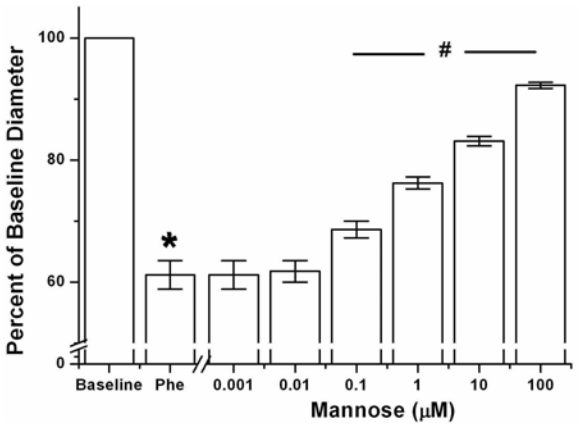

Figure 2. D-Mannose intraluminal infusion induces vasodilation on pre-contracted rat mesenteric arteries. Changes in mesenteric artery diameter were expressed as percent of baseline diameter. Rat mesenteric arteries were pre-contracted with phenylephrine 10μmol/L (Phe). Vasodilation induced by D-mannose intraluminal infusion is concentration-dependent. Data are expressed as mean±SE. * and # compared vs. Baseline diameter, and were statistically different, $p<0.05$.

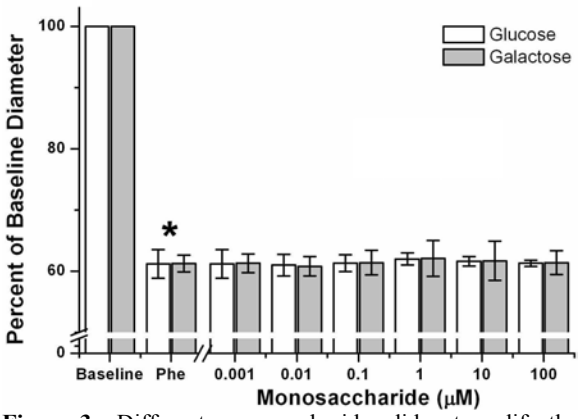

Figure 3. Different monosaccharides did not modify the vascular tone on pre-contracted rat mesenteric arteries. Changes in mesenteric artery diameter were expressed as percent of the baseline diameter. Rat mesenteric arteries were pre-contracted with phenylephrine 10μmol/L (Phe). Neither D-glucose nor D-galactose modified the vascular tone on the pre-contracted rat mesenteric arteries. Data are expressed as mean±SE. * compared vs. Baseline diameter, and was statistically different, $p<0.05$.

detect the mRNA expression of the cation-dependent mannose receptor on rat mesenteric arteries, we developed a LightCycler PCR assay using the TaqMan probe with the condition described in the materials and methods. We made three repetitions in different times and used the liver of the rat as positive control and the mesenteric artery and a negative control. The liver sample and mesenteric arteries showed amplification curves and the negative control was not amplified (fig. 6). With these results, we verified the presence of the cation-dependent mannose receptor in the rat mesenteric arteries.

4.4. FITC-mannose-polymer perfusion remains in the vascular lumen

These results suggested that the mannose-induced effects might be exerted trough the stimuli of specific receptors, however did not allow us to discriminate the site of action. To explore the possible site of action, we used a FITC-mannose-polymer, our results shown that in fact the polymer remains confined to the vascular lumen (fig. 7) suggesting that the endothelium is the main site of mannose stimuli.

The possible participation of nitric oxide or prostaglandins in the mannose-induced effects was explored as mediators of vasodilation mechanism. Nitric oxide synthase inhibitor L-NAME [10μmol/L] and indomethacin [10μmol/L] as COX inhibitor were used and rat mesenteric arteries were incubated 15min after phenylephrine administration, previous and during D-mannose polymer perfusion. Neither the nitric oxide synthase inhibitor nor the COX inhibitor was effective on the inhibition of the vasodilation of the free D-mannose or mannose polymer. It was also assayed the effects of reactive Blue (1μmol/L), glibenclamide (1μmol/L), apamin (10μmol/L), aminophylline (10μmol/L), purinoceptor blocker, K_{ATP} and K_{Ca} channels blockers, adenosine receptor antagonist, superoxide dismutase scavenger and antioxidant, respectively, (data not shown); unfortunately, at this point no mediator of the mannose polymer induced vasodilation is not yet to be elucidated.

5. DISCUSSION

D-Mannose is a single epimerized carbohydrate from D-glucose at the two position. This apparent slight structural difference between these two essential sugars confers each other specific functions and metabolic pathways (16,36). At the cellular level, D-mannose is used for the biosynthesis of oligosaccharides and glycophospholipid anchors. Under normal circumstances, it does not appear to make a large contribution to general energy metabolism (37). Several studies have shown that D-mannose can be converted into glycogen but this is under extreme over dietary mannose ingestion. It is metabolized like glucose at cellular and organism level (37). The plasma mannose presumably comes from a combination of dietary sources, normal oligosaccharides processing and turnover of endogenous glycoproteins and free oligosaccharides (38). D-Mannose residues have a key role in the proper folding for a large number of proteins synthesized by the ER, and also for their proper transport to the Golgi, for secretion or membrane translocation (39). Mannosylation of microorganisms cell surface proteins induces the innate immune response through the proper and specific recognition of these carbohydrate residues by several mammal's lectins (40,41). Alternative complement activation involves the binding of D-mannose residues to a soluble mannose binding lectin (MBL) as the start of the pathway cascade (42,43,44,45). Also, membrane lectins recognition of D-mannose residues mediates the activation of intracellular signal pathways, such as those that internalize mannosylated proteins like asialoglycoprotein or some glycosylated proteins and enzymes that have been

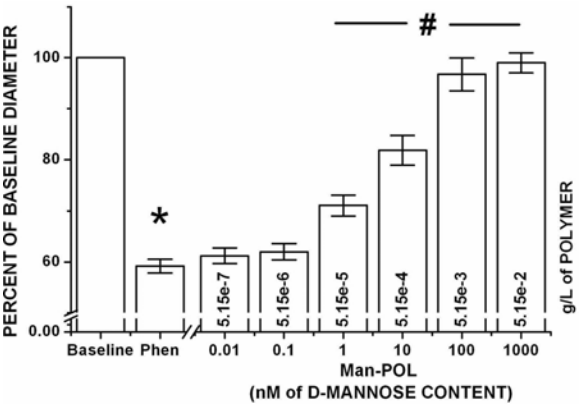

Figure 4. Mannose-polymer infusion induces a more potent vasodilation on pre-contracted rat mesenteric arteries. Changes in mesenteric artery diameter were expressed as percent of the baseline diameter. Rat mesenteric arteries were pre-contracted with phenylephrine 10μmol/L (Phe). Concentrations on the X-axis are expressed according with the mannose content of the mannose polymer (3.5 mg of mannose per g of polymer). Numbers inside the bars indicate the amount of Mannose polymer in g/L in order to compare the effect with dextran 260,000 Da effect. Data are expressed as mean±SE. * and # compared vs. Baseline diameter, and were statistically different, $p<0.05$.

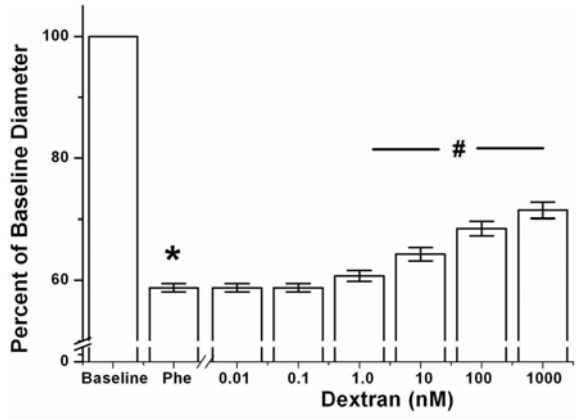

Figure 5. A dextran 260,000 Da infusion induces a marginal vasodilation on pre-contracted rat mesenteric arteries. Changes in mesenteric artery diameter were expressed as percent of the baseline diameter. Rat mesenteric arteries were pre-contracted with phenylephrine 10μmol/L (Phe). Higher concentration of dextran 260,000 Da only induced a slightly vasodilation on rat mesenteric arteries, viscosity values are not significantly increased to explain the vasodilatory effect. Data are expressed as mean±SE. * and # compared vs. Baseline diameter, and were statistically different, $p<0.05$.

processed by some exoglycosidases exposing D-mannose residues (17,46,47,48).

D-mannose residues in polymers of synthetic origin seem to active other signal pathways that induced an enhancement of the positive inotropism and dromotropism mediated by the coronary flow in isolated guinea pig hearts through a mannose binding lectin at endothelial membrane level (27). Previous report showed that membrane mannose receptor increases the up-take effect directly proportional to the polymerization of mannose (25). Our findings show; first, that intraluminal perfusion of free D-mannose, or synthetic high molecular mannose polymer (> 260 kDa) induces concentration-dependent vasodilation of pre-contracted, pressurized rat mesenteric arteries. The mannose polymer induced vasodilation is 100 times more potent than free D-mannose, this difference between free mannose and mannose polymer could suggest that the effect induced by the mannose polymer could be mediated by a MR-like structure in the surface of the endothelium of rat mesenteric arteries as previous studies shows (25,27). Second, the vasodilation effect is a specific effect for this monosaccharide, because there was no effect by D-glucose or D-galactose intraluminal perfusion on pre-contracted, pressurized rat mesenteric arteries. Third, dextran has been used to modify perfusion solution viscosity, and therefore shear stress, an important vascular tone modulator; dextran is a polysaccharide constituted by glucose residues only (glucose α1-6 glucose). Dextran 260,000 Da concentrations perfused did not increase significantly the viscosity of the perfusion solution; dextran intraluminal perfusion induces a small concentration-dependent vasodilation on pre-contracted, pressurized rat mesenteric arteries. However, dextran-induced effect is less potent than the vasodilation effect induced by the intraluminal perfusion of mannose polymer, and this effect is not an additive effect by D-mannose and dextran, because simultaneous intraluminal perfusion of free D-mannose and dextran higher concentrations did not significantly increase vasodilation effect (data not shown). Gonzalez-Castillo et al (49) tested several sizes of dextran in isolated and perfused guinea pig hearts and they showed that dextran perfusion modified the myocardial contraction and this effect was independent of the perfused solution viscosity. They showed that dextran 260,000 Da increases viscosity marginally in the range of the concentrations that we tested in this study (49). Fourth, our findings with the intraluminal perfusion of fluorescent labeled-mannose polymer suggest that vasodilatory effect on rat mesenteric arteries seems to be mediated by the presence of a mannose receptor at luminal endothelium membrane, furthermore macrophage mannose receptor mRNA was found in rat mesenteric arteries. It has been reported that the exclusion size of dextran is 70,000 Da (7). Based on this report and in our results, we propose that the main site of MR activation or at least site of mannose interaction with luminal-related molecules in the mesenteric arteries is the endothelial surface layer. Figure 7 demonstrated that the fluorescent labeled-mannose polymer remains confined to the luminal space. Fifth, at this point vasodilatory mediator has not been elucidated, common vasodilators such as NO and COX-derivatives were ruled out to mediate mannose polymer-induced vasodilation on pre-contracted pressurized rat mesenteric arteries.

Mannose receptor (MR) is a carbohydrate pattern recognition receptor expressed mainly on cells of myeloid lineage, with few exceptions. Besides some specialized

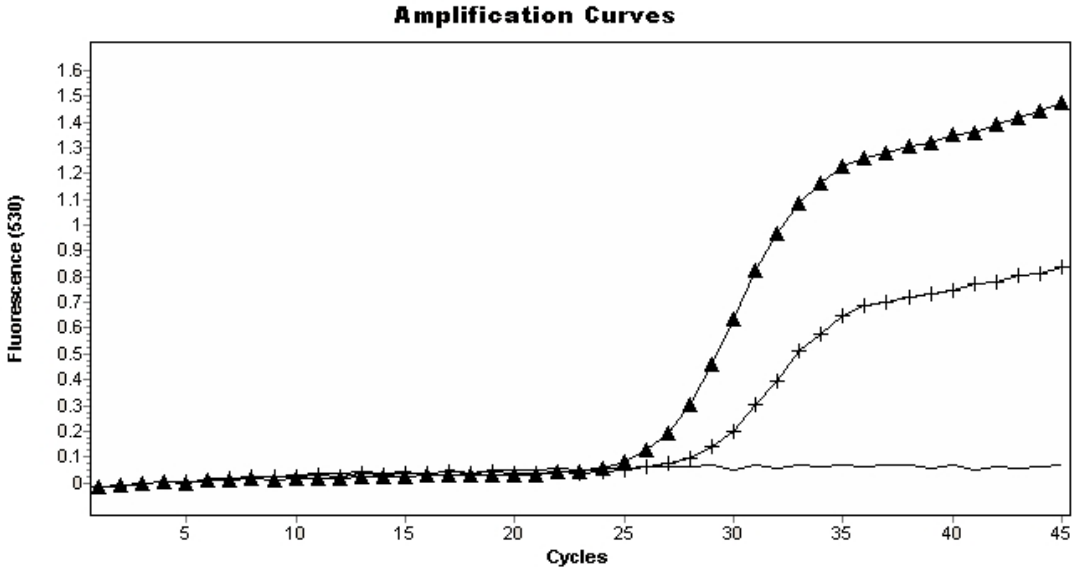

Figure 6. RT-PCR of mannose receptor mRNA in rat mesenteric arteries. Real-Time PCR reactions were conducted using the Rat Universal Probe Library (Roche Diagnostics GmbH, Basel Switzerland). The primer sequences of liver MR were forward 5'AGCCACTCCGAACCTGGCAGC3', and reverse 5'TCATGCGACATGGGTTCCTGG3'. Triangles show the positive control (liver), mRNA amplification of mesenteric arteries is shown in (+) curve.

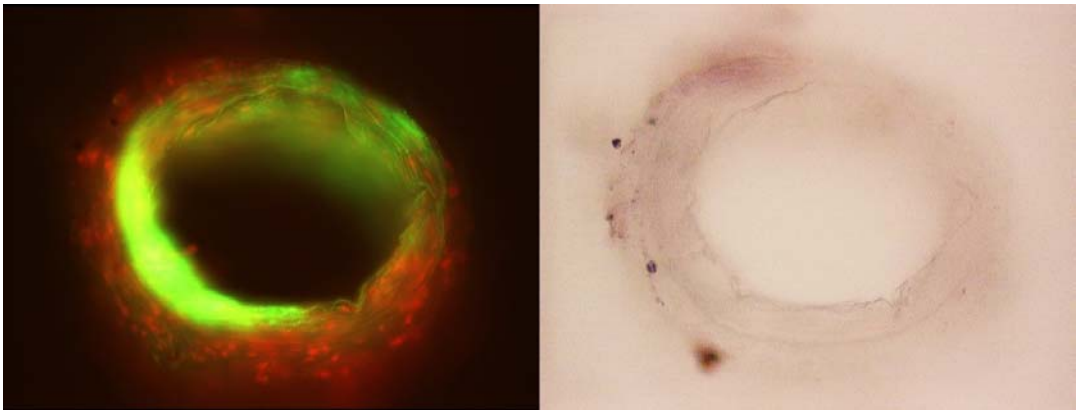

Figure 7. FITC-Mannose polymer labels on the endothelial cell surface of the rat mesenteric arteries. A 1mm segment of rat mesenteric artery infused with FITC-Mannose polymer (green) for 5min and extensively washed was mounted with Vectashield with propidium iodide (red). Left image shows the rat artery visualized with DIC. Right image shows that the FITC-mannose polymer (green) binds to the luminal surface of the rat mesenteric artery; propidium iodide (red) labels nuclei of smooth muscle cells (in circular pattern external of the artery lumen). Bar indicates 100μm.

cell types in the kidney, trachea and retina, MR has only been described on the endothelial cells of organs which are specialized in antigen uptake/clearing and/or presentation such as the liver, spleen and lymph nodes (18,20,21,47). MR also mediates endocytosis and phagocytosis (9,25,46,48,50,51,52,53,54,55). This receptor is unusual among C-type lectins in having multiple CRDs in a single polypeptide chain; it also has an extracellular region consisting of an N-terminal cysteine-rich domain followed by fibronectin type II (16,17). MR binds the monosaccharide mannose, fucose and N-acetylglucosamine, but displays much higher affinity for the multivalent oligosaccharides, such as those found on the surface of potentially pathogenic microorganism and mannan like structures (56,57). D-Mannose binding is Ca^{2+}-dependent in the CRD. At least three CRDs (4,5 and 7) are known to be required for high affinity and binding to multivalent ligands. The pH sensitivity of Ca^{2+} binding differs between isolated CRD-4 and larger fragments of the receptor, which implies that inter-CRD interactions in the intact mannose receptor can tune the pH sensitivity of ligand binding.

Mannose receptor releases its bound ligand in early endosomes and recycles to the cell surface. The endocytic vesicle containing the receptor-ligand complex

becomes progressively more acidic, resulting in titration of Ca^{2+} off the CRD and loss of the ligand. MR or mannose binding structures seem to be involved in other vascular-related effects. In normal skin specimens MR expression was found on ~30-50% of small and medium-sized blood vessels of the deep vascular plexus of the dermis. All MR-positive vessels coexpressed CD36 molecules (20). In the recent past, we have shown that the infusion of mannose and mannose polymer in isolated and perfused guinea pig hearts induces a positive inotropism as a result of binding to the endothelial surface and interaction with lectin-like proteins on the luminal endothelial cell surface of the coronary vasculature (27). Macrophage mannose receptor may be a signal transducing receptor triggering a variety of responses including secretion of mediators, lysosomal enzyme secretion, cytokine production, and modulation of other cell surface receptors.

Recently, it was reported that activation of MR on macrophages induced increase in prostaglandin production and COX expression (26), we found no relation of mannose-induced effects with nitric oxide, prostaglandin, adenosine receptors, P_{2Y} purinoceptors, K_{ATP} channels and antioxidants. In fact, we were unable to find the mediator of mannose and mannose polymer-induced effects, these might be related with electrochemical myo-endothelial communication as it has been proposed by Figueroa et al (58,59). Another pathway that might be involved in the vasodilatory effect of the mannose polymer is the change in the pH; as mention above in order to release mannose residue from MR pH must be modified to increase dissociation rate. The changes in extracellular and intracellular pH (pH_o and pH_i, respectively) have been shown to modulate vascular contractility by affecting the activities of ion channels and pumps (39). H+ may interact with voltage-operated Ca2+ channels both internally and externally (12,43). These effects of H+ on Ca2+ and K+ channels could clearly account for the decrease in intracellular Ca2+ concentration and also for the decrease in contractility on acidification. On the other hand, decreases in pHi and pHo inhibited inwardly rectifying K+ (KIR) currents (3, 16, 60). However, more work is necessary to elucidate the mechanism behind the vasodilatory effect of mannose polymer.

In conclusion, the mannose receptor is expressed and functional in rat mesenteric arteries. Its activation induces a vasodilatory effect in pre-contracted arteries unrelated to nitric oxide, COX derived molecules, adenosine receptors, P_{2Y} purinoceptors and K_{ATP} or K_{Ca} channels. MR activation seems to be located on the luminal surface of the endothelium and the function is unrelated to immunity or clearance. The observed effects might be of physiological relevance because the assayed D-mannose concentrations were in the physiological range of mannose in the blood [20-50µmol/L] (60).

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