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Caveolin: A possible biomarker of degradable metallic materials toxicity in vascular cells ☆

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ABSTRACT

Iron-based materials could constitute an interesting option for cardiovascular biodegradable stent applications due to their appropriate ductility compared with their counterparts, magnesium alloys. However, the predicted degradation rate of pure iron is considered to be too slow for such applications. We explored manganese (35 wt.%) as an alloying element in combination with iron to circumvent this problem through powder metallurgical processing (Fe–35Mn). Manganese, on the other hand, is highly cytotoxic. We recently explored a new method to better characterize the safety of degradable metallic materials (DMMs) by establishing the gene expression profile (GEP) of cells (mouse 3T3 fibroblasts) exposed to Fe–35Mn degradation products in order to better understand their global response to a potentially cytotoxic DMM. We identified a number of up- and down-regulated genes and confirmed the regulation of a subset of them by quantitative real time polymerase chain reaction. Caveolin-1 (cav1), the structural protein of caveolae, small, smooth plasma membrane invaginations present in various differentiated cell types, was one of the most down-regulated genes in our GEPs. In the present study we further studied the potential of this 22 kDa protein to become a biomarker for cytotoxicity after exposure to degradable metallic elements. In order to better characterize cav1 expression in this context 3T3 mouse fibroblasts were exposed to either ferrous and manganese ions at cytostatic concentrations for 24 or 48 h. cav1 gene expression was not influenced by exposure to ferrous ions. On the other hand, exposure to manganese for 24 h reduced cav1 gene expression by about 30% and by >65% after 48 h compared with control 3T3 cells. The cav1 cellular protein content was reduced to the same extent. The same pattern of expression of cav3 (the muscle-specific caveolin subtype) was also observed in this study. This strong and reproducible pattern of regulation of caveolins thus indicates potential as a biomarker for the toxicity of DMM elements.

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1. Introduction

Caveolae (little caves) were first described as flask-shaped plasma membrane invaginations capable of transporting molecules across the endothelial barrier. They are abundant, mostly in highly differentiated cell types such as endothelial and smooth muscle cells [1]. Caveolae have been associated with a number of cellular functions or processes, such as potocytosis, cholesterol homeostasis, transformation, and the control of signal transduction [2,3]. Their functions are related to their structure, being rich in cholesterol, sphingomyelin and glycosphingolipids. Caveolins, the biochemical marker for caveolae, also play a central structural role in their formation. The caveolin family comprises at least three genes that are expressed heterogeneously in different cell types. Caveolin-3 (cav3) is specifically expressed in muscle cells [4,5], while caveolin-1 (cav1) and caveolin-2 (cav2) are expressed in most cell types, such as fibroblasts, adipocytes, endothelial cells, and pneumocytes [6].

The implantation of a metallic stent in an artery is associated with mechanical disruption of the endothelial layer. Using biodegradable metallic stents could also result in additional damage to the surrounding tissue due to the release of degradation end-products. cav1 has recently been reported to be a potential biomarker of vascular injury caused by certain vasodilators [7]. cav1 is highly expressed in normal blood vessels but its expression is decreased at sites vascular injury. This raises the possibility that cav1 expression could be a marker of vascular injury at the site of implantation

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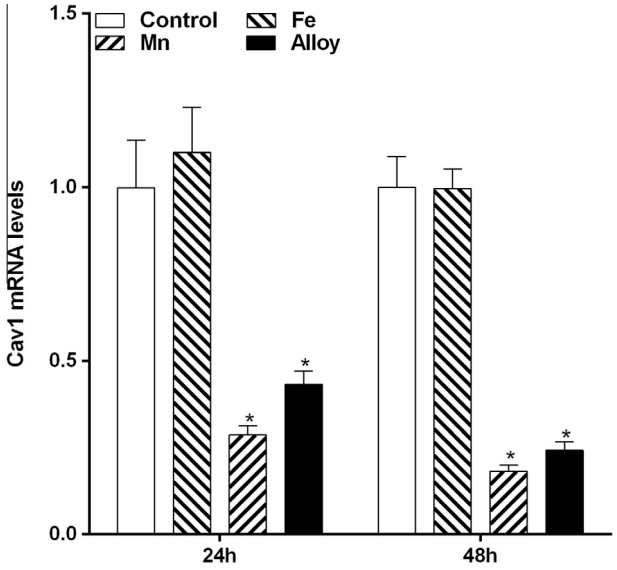

Fig. 1. Caveolin-1 (cav1) mRNA levels in 3T3 fibroblasts after a 24 or a 48 h exposure to powdered alloy, Mn, or Fe. The maximum diameter of the powder was 75 μm. The powder was added to a 3 μm tissue culture insert. Fibroblasts were pre-cultured overnight prior to metal exposure. The results are expressed as means ± SEM ($n = 6$) relative to mRNA levels measured in controls (arbitrarily fixed at 1). $^{*}p < 0.001$ vs. control.

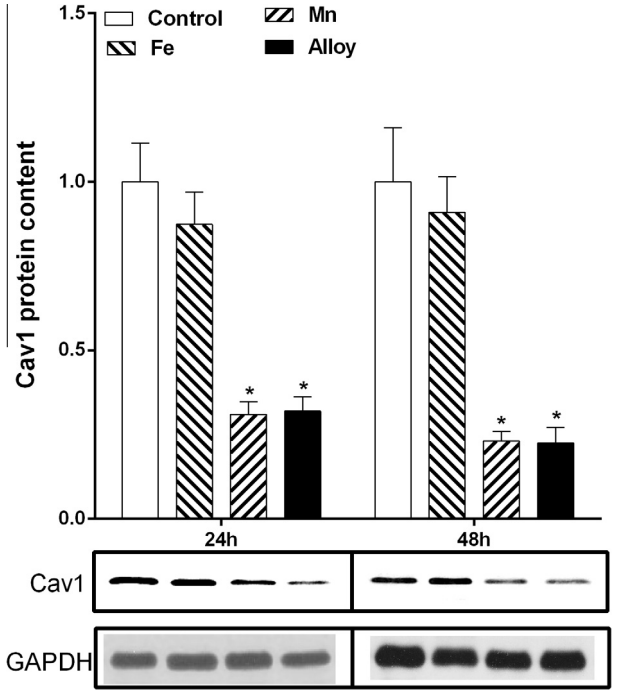

Fig. 2. Caveolin-1 (cav1) protein content in 3T3 fibroblasts after a 24 or a 48 h exposure to powdered alloy, Mn, or Fe. The results are expressed as means ± SEM ($n = 6$) relative to protein content measured in controls (arbitrarily fixed at 1). $^{*}p < 0.001$ vs. control. A view of representative results from the caveolin-1 immunoblot for each treatment group is illustrated at the bottom of the graph. The 22 kDa unique band can be seen.

of a stent. cav1 also acts as a binding site of nitric oxide synthase (NOS), which is responsible for nitric oxide production [8].

We recently reported that cav1 expression was decreased in a cultured fibroblastic cell line when exposed to the degradation products of degradable metallic materials (DMMs) [9]. In fact,

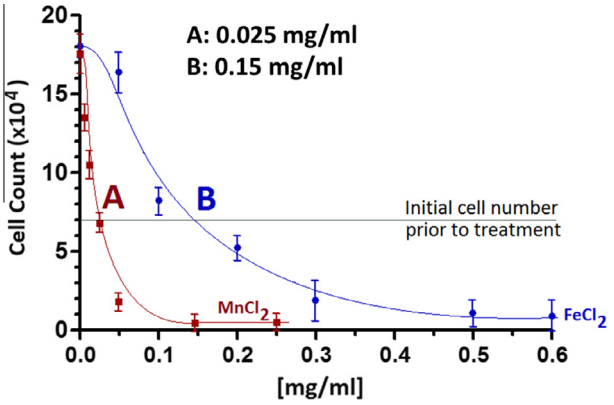

Fig. 3. Fibroblast cell count after a 24 h exposure time with increasing concentrations of either $FeCl_2$ or $MnCl_2$. Fibroblasts were pre-cultured overnight prior to metal salt exposure. Cells were counted under a light microscope using a hemocytometer. The results are expressed as means ± SEM ($n = 6$).

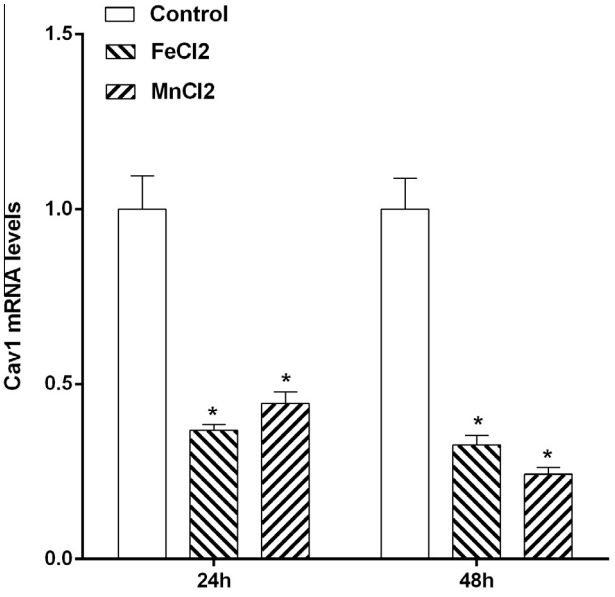

Fig. 4. Caveolin-1 (cav1) mRNA levels in 3T3 fibroblasts after a 24 or a 48 h exposure to $FeCl_2$ (0.15 mg ml^{-1}) or $MnCl_2$ (0.025 mg ml^{-1}). Fibroblasts were pre-cultured overnight prior to metal exposure. The results are expressed as means ± SEM ($n = 6$) relative to mRNA levels measured in controls (arbitrarily fixed at 1). $^{*}p < 0.001$ vs. control.

earlier experiments with 3T3 fibroblasts showed that cav1 was down-regulated in the presence of DMM and that this pattern of expression was observed as early as 24 h after exposure to Fe–35Mn alloy and was stable up to 48 h exposure. It is generally believed that the presence of DMMs is associated with the release of metallic ions that act as oxidants and lead to a charge imbalance within the surrounding tissue [10]. Our findings have shown that the expression of cav1 is decreased in the presence of DMMs, which is in agreement with other reported results where ozone was applied as an oxidant in a lung injury mouse model [11]. In this study we further studied the expression pattern of caveolins when cells are exposed to DMMs.

2. Materials and methods

2.1. Cell culture

BALB/3T3 mouse fibroblast cells (ATCC no. CL-163, clone A31) were cultured in Dulbecco's modified Eagle's medium (DMEM)

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