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Leading opinion

Cell based metabolic barriers to glucose diffusion: Macrophages and continuous glucose monitoring

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ABSTRACT

It is assumed that MQ are central to glucose sensor bio-fouling and therefore have a major negative impact on continuous glucose monitoring (CGM) performance *in vivo*. However to our knowledge there is no data in the literature to directly support or refute this assumption. Since glucose and oxygen (O_2) are key to glucose sensor function *in vivo*, understanding and controlling glucose and O_2 metabolic activity of MQ is likely key to successful glucose sensor performance. We hypothesized that the accumulation of MQ at the glucose sensor-tissue interface will act as "*Cell Based Metabolic Barriers*" (CBMB) to glucose diffusing from the interstitial tissue compartment to the implanted glucose sensor and as such creating an artificially low sensor output, thereby compromising sensor function and CGM. Our studies demonstrated that 1) direct injections of MQ at *in vivo* sensor implantation sites dramatically decreased sensor output (measured in nA), 2) addition of MQ to glucose sensors *in vitro* resulted in a rapid and dramatic fall in sensor output and 3) lymphocytes did not affect sensor function *in vitro* or *in vivo*. These data support our hypothesis that MQ can act as metabolic barriers to glucose and O_2 diffusion *in vivo* and *in vitro*.

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1. Introduction

The currently approved usage lifespans for commercial continuous glucose monitor (CGM) *in vivo* ranges from 3 days to 7+ days. Achieving euglycemia with an artificial pancreas requires a highly accurate CGM. Inflammation is a significant complication of CGM, but the nature of this inflammation and the mechanism involved are not well understood. It is universally accepted that macrophages (MQ) are a histologic hallmark of chronic inflammation including foreign body reactions [1]. Implantable glucose sensors used in CGM of diabetic patients are known to induce foreign body reactions characterized by accumulation of macrophages (MQ) at the sensor-tissue interface. Since MQ are not only pivotal in mediating inflammation as well as wound healing, it is critical to define the role of these cells and their products utilizing biosensor function *in vivo*. The paucity of information regarding the mechanistic role of MQ, MQ subpopulations and their products in controlling sensor function *in vivo* represents a critical gap in our knowledge.

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Our laboratory previously reported that red blood cells (RBC) serve as a consumptive barrier for glucose sensors by creating "metabolic sinks", which consume glucose when pooled around glucose sensors in clot formation (hemorrhages) [2,3]. In these studies, we concluded that RBC accumulation at glucose sensor sites resulted in a marked decrease in local glucose levels. This localized RBC glucose consumption at the site led to a significant reduction in sensor output. Thus, this RBC-based "metabolic sink" mimicked the loss of sensor function when the sensor was correctly reporting the reduced glucose levels in the surrounding microenvironment. Simulation studies reported by Novak et al. [4] concluded that inflammatory cells but not RBC at site of sensor location were responsible for increased glucose consumption and the observed "anomalous" sensor behavior. There is no dispute that inflammatory cells are glucophagic. However, we opine that the "anomalous readings" are better explained by the micro-hemorrhage induced by sensor implantation such that the RBC are responsible for the majority of the glucose consumption when submerged in whole blood. At initial insertion time, the erythrocyte to leukocyte ratio (mainly polymorphonuclear neutrophils (PMN)) is about 1000:1. Nonetheless, erythrocytes in the tissue as a result of hemorrhage are less metabolically active over time as compared to leukocytes. In addition, inflammatory cell removal of RBC from the hemorrhages adjacent to the sensor site, likely increases the

degree of sensor-associated inflammation with a resultant decrease in sensor function. Thus, it would be difficult to predict the ratio between RBC and inflammatory cells in this environment. This may be further complicated by hemorrhage formation due to sensor movement hours or days post sensor implantation when a significant number of inflammatory cells are already present at the sensor site.

Notwithstanding, micro-hemorrhages around glucose sensors do not invariably occur. In contrast, accumulation of inflammatory cells characterized by MQ recruitment and accumulation at the sensor-tissue interface are almost invariably seen at sensor implantation sites [5]. This occurs with both acute and chronic inflammatory processes including foreign body reactions. Moreover, macrophages are highly metabolically active cells that consume significant quantities of glucose and oxygen in order to generate their responses to tissue injury and invasive pathogens [6]. We recently reported that MQ accumulate at the sensor-tissue site over time and form a barrier that surrounds the implanted sensor with resultant impairment of continuous glucose sensor performance [5]. This publication also reported that MQ deficient or depleted animals demonstrated enhanced sensor performance [5].

These studies and the observations that MQ are highly metabolically active support our hypothesis that the recruitment and accumulation of MQ at the sensor-tissue site create "*Cell Based Metabolic Barriers*" (CBMB) to glucose, which results in impaired glucose sensor performance (see Fig. 1). Additionally MQ consume significant amounts of oxygen as part of their anti-microbial functions. MQ induced local oxygen consumption occurs in the production of superoxides, which can adversely affect oxygen dependent glucose sensors [7,8] (see Fig. 1). This was assessed with an *in vivo* murine model of CGM that characterized MQ-sensor interactions. MQ impacts on glucose sensors *in vitro* were also assessed with newly developed *in vitro* cell culture based system. The studies demonstrated the following: direct injections of MQ at sensor implantation sites decreased sensor function as represented by the fall in sensor output; addition of MQ to glucose sensors *in vitro* resulted in a rapid reduction in sensor output and that lymphocytes did not affect sensor function *in vitro* or *in vivo*. These data support our hypothesis that the accumulation of MQ at the sensor-tissue interface acts as a metabolic barrier to glucose diffusion from the interstitial tissue compartment to the implanted glucose sensor (Fig. 1). This process results in an artificially low sensor output, which compromises CGM. These data could be incorporated into future therapeutic interventions and new sensor designs that ought to lead to acceptable sensor performance and CGM accuracy.

2. Materials and methods

2.1. Murine continuous glucose monitoring (CGM): glucose sensors, implantation and mice

All modified Navigator glucose sensors used in these *in vivo* studies were obtained from Abbott Diabetes Care. Glucose sensors were implanted into mice and continuous glucose monitoring (CGM) was undertaken as previously reported [9]. Implanted sensors were secured to the mouse skin with a mesh, and CGM was initiated per protocol [9]. Blood glucose reference measurements were obtained at least daily using blood obtained from the tail vein of the mouse and a FreeStyle® Blood Glucose Monitor. All C57BL/6J mice used in these studies where obtained from Jackson Laboratories, Bar Harbor Maine. The Institutional Animal Care and Use Committee of the University of Connecticut Health Center (Farmington, CT) approved all the studies involving mice.

2.2. Macrophage injections at glucose sensor implantation sites

To investigate the ability of MQ to suppress glucose sensor function *in vivo* isolated mouse macrophages were directly injected at the sensor implantation site in normal C57BL/6 mice (Fig. 2). Specifically, thioglycolate induced peritoneal MQ from normal C57BL/6 mice were obtained as previously described [10]. Glucose sensors were implanted and after a sensor run-in time of about 24 h either saline or peritoneal MQ were directly injected at site of sensor location. For these studies 10^5-10^7 MQ per injection site were used (arrow in Fig. 2). To determine "cell specificity/MQ dependence" of these reactions mouse spleen-derived lymphocytes were also tested in place of MQ at sites of sensor implantation.

2.3. Histopathologic analysis of tissue reactions at glucose sensor implantation sites

In order to evaluate the tissue responses to macrophage injections at glucose sensor implantation sites, individual mice were euthanized and the tissue containing the implanted sensors was removed, fixed in 10% buffered formalin for 24 h, followed by standard processing, embedded in paraffin and sectioned. The resulting 4–6 um sections were then stained using standard protocols for hematoxylin/eosin stain (H/E). The tissue samples were examined for signs of inflammation, including necrosis, fibrosis, and vessel regression. Resulting tissue sections were evaluated directly and documented by digitized imaging using an Olympus Digital Microscope.

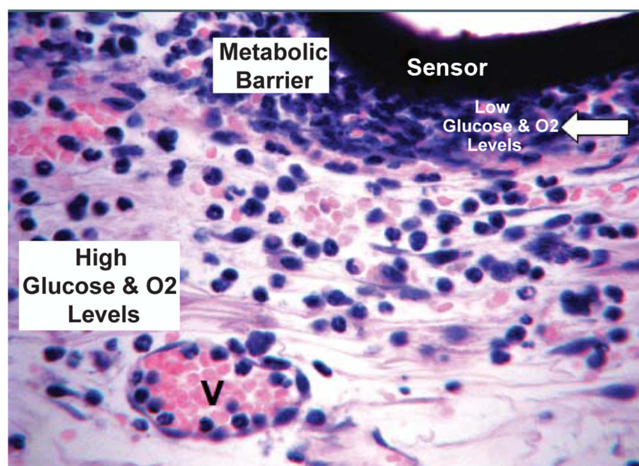

Fig. 1. Monocyte related cells create "*cell based metabolic barriers*" (CBMB) to glucose and oxygen diffusion at sites of glucose sensor implantation and compromise CGM. Based on the literature and recent data from our laboratories, we hypothesized that the recruitment and accumulation of monocyte related cells (i.e. MQ and FBGC) at the sensor-tissue interface create a "*Cell Based Metabolic Barrier*" (white arrow) to glucose and oxygen that is diffusing from the vasculature (V) toward implanted glucose sensors (sensor).

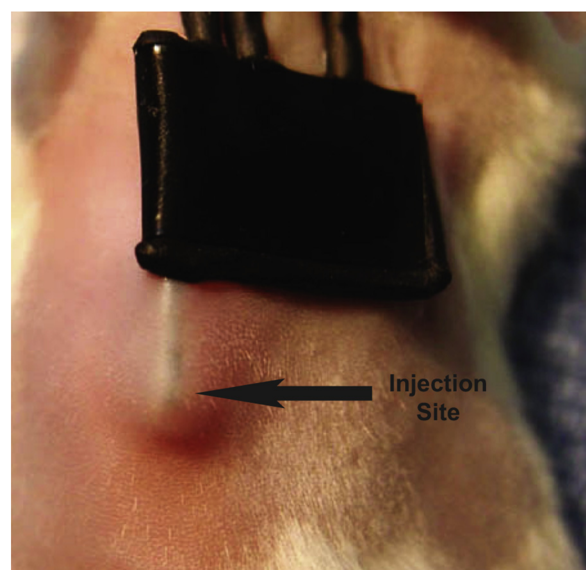

Fig. 2. Cell injection site at glucose sensor implantation site in murine model of CGM. For *in vivo* cell injection studies, cells (MQ or spleen lymphocytes) we injected subcutaneously on the back of mice (red arrow) used in our murine model of CGM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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