Journal of Electrocardiology 55 (2019) 72–77

Contents lists available at ScienceDirect

Journal of Electrocardiology

journal homepage: www.jecgonline.com

Potential mechanisms of endothelialisation in individuals implanted with a leadless pacemaker systems: An experimental in vitro study

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ARTICLE INFO

Keywords:
Leadless pacemaker
Micra system
Material composition
Inflammation
IL-6
IL-1β
IL-8

ABSTRACT

Background: Leadless pacemaker technology is a promising upcoming field in clinical rhythmology. Today, the most commonly used system in the clinical setting is the Micra™ leadless pacemaker system (Medtronic). In autopsies of patients who witnessed non-pacemaker associated death, unexpected ingrowth/encapsulation within the wall of the right ventricle was reported. The occurrence of a complete encapsulation was not expected and the process of endothelialisation remains unclear. We hypothesized, that a local inflammatory response might be the cause of these findings. The aim of our experimental in-vitro study was to investigate the effect of the Micra™ system and its single components on inflammatory processes.
Methods: For this purpose, whole Micra™ pacemakers were incubated in heparin plasma from 25 healthy volunteers for 48 h at 37 °C. Furthermore, 1 g gold, steel, titanium, tungsten and nitinol wires were incubated in heparin plasma for 48 h at 37 °C as well ($n = 10$).
To detect eventual inflammatory processes, interleukin- (IL) 1β, IL-6, and tumor necrosis factor alpha (TNF-α), the chemokine IL-8 were measured using enzyme-linked immunosorbent assay (ELISA). Additionally, the level of transforming growth factor beta 1 (TGF-β1) and vascular endothelial growth factor (VEGF) were analysed.
Results: ELISA analyses showed that the whole Micra system leads to a significant increase in the inflammatory cytokine IL-6 which correlates with the data gained by the incubation of whole blood with the different wires. In particular, 0.5 g of tungsten showed a significant rise of IL-6 which could also be found for IL-1β and IL-8.
Conclusions: The in vitro study of the Micra system showed that the material composition led to an onset of inflammatory processes in whole blood. Consequently, one may speculate that the composition of Micra pacemaker may have a local inflammatory, though subclinical, effects in patients implanted with a Micra™ pacemakers.

Introduction

The only evidence based treatment option for patients suffering from symptomtic Bradycardia is the implantation of a permanent cardiac pacemaker device.

Nowadays, a standard system consists of two components, the pacemaker itself with its integrated battery and electronics, which usually is implanted into a subcutaneous pocket, and one or more leads. Even though these conventional devices have strong evidence to work safe and effective, a not negligible amount of patients encounter complications associated with their device.

Leads showing any kinds of functional loss, damage or infection have to be removed in many cases. Unfortunately, a surgical lead extraction is usually challenging and has to be done in a highly experienced centre with the possibility to perform sternotomia in case of a sever complication such as pericardial tamponade [1].

Due to the progress in technical possibilities, a completely new generation of pacemaker devices have been developed, which are placed intracardially and therefore do no longer require any leads at all. Currently, there are two different types of these leadless pacemaker-devices available [2,3]. The Micra™ (Medtronic Inc., Minneapolis, MN,

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https://doi.org/10.1016/j.jelectrocard.2019.04.012
0022-0736/

USA) transcatheter pacing systems is currently the by far more used in the clinical setting. It is a 0.8 cubic centimeter, 2.0 g capsule with a length of 25.9 mm and an outer diameter of 6.7 mm with the features of a single-chamber pacemaker system. Implantation into the right ventricle is performed by a steerable transfemoral catheter delivery system, which requires a 23 French introducer [4]. The second currently available device is the Nanostim™ (Abott Inc., Santa Clara, California, USA former: St. Jude Medical Inc., Saint Paul, Minnesota, USA) transcatheter pacing system [3].

Moreover, several clinical cases could demonstrate the leadless technology to be an effective alternative treatment option in patients, who suffered from a systemic infection of their conventional device [5,6]. According to the manufacturers, both devices are expected to remain floating within the inner heart, being connected to the endothelium with their anchor systems only.

Since the leadless technology is an upcoming new therapeutic option, the impact on the endothelium at the fixation-site is currently not well known. The knowledge, how the focal endothelial reaction will look like, is of clinical importance to evaluate whether a replacement of the system in case of damage or end of service would still be feasible. However, in both devices unexpected cases of encapsulation were reported, which could be documented during autopsies in patients, who witnessed non-pacemaker associated death.

Several cases showed partial or even complete encapsulation [7,8]. Another published case describing this phenomenon was a patient suffering from chronical obstructive lung disease, who underwent leadless implantation due to permanent atrial fibrillation with symptomatic bradycardia [9].

The purpose of our in vitro study was whether single components or even the whole system would lead to a measurable increase in

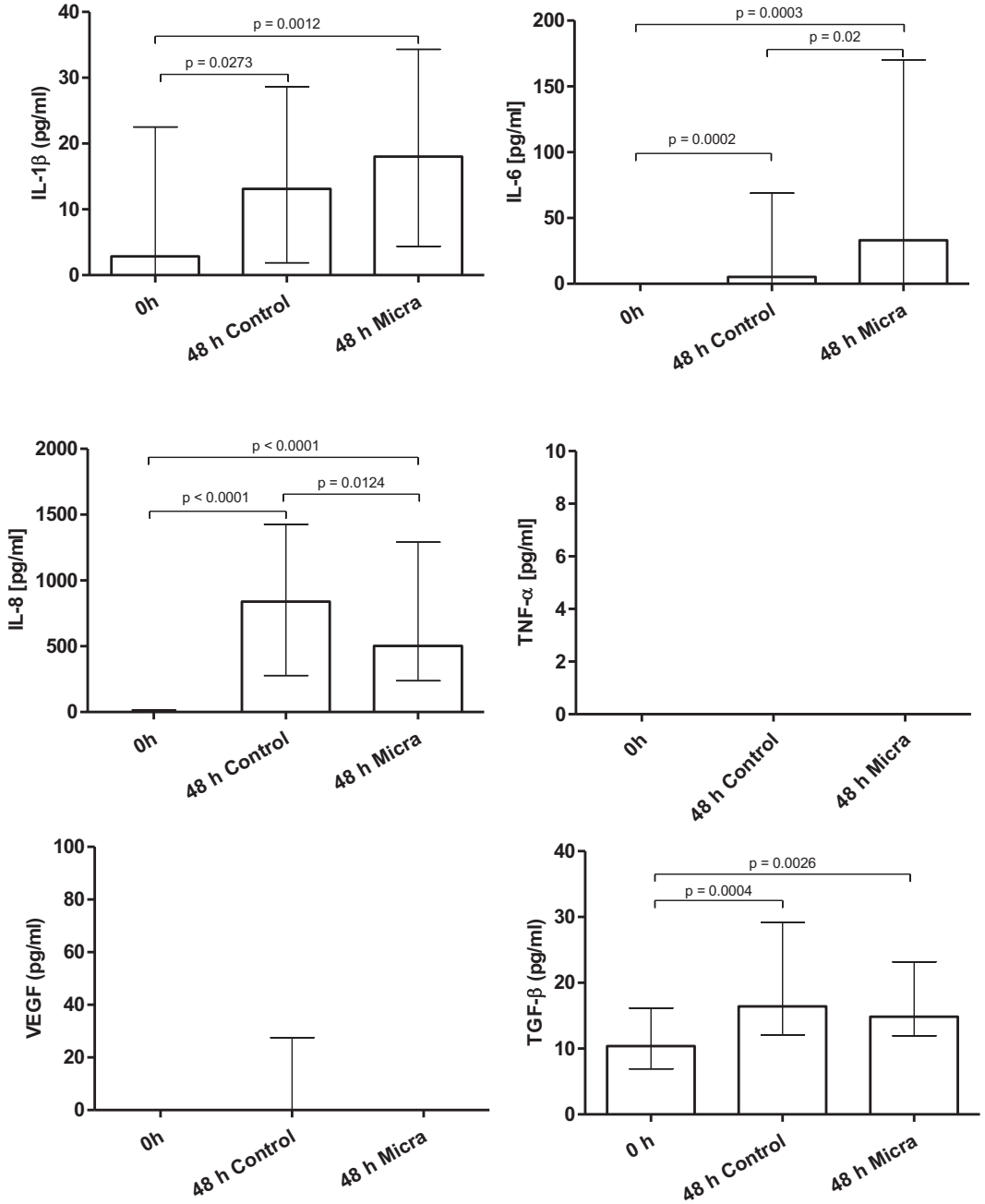

Fig. 1. Effects of incubation of the Micra system in heparinized whole blood. IL-6 showed a significant increase and IL-8 a significant decrease compared to controls, while TNF-α and VEGF did not shown relevant differences. IL-1β evidenced a slight, but significant, increase and TGF-β a slight decrease.

inflammatory cytokines as a potential explanation for these unexpected findings.

Materials and methods

All devices used in this experimental setting were prior in clinical use and explanted after non pacemaker associated death of the patient at the University hospital in Linz.

Blood sample preparation

To examine the influence of the whole Micra pacemakers' material composition on inflammatory factors, blood was drawn from 25 healthy volunteers from cubical vein using a clean venipuncture. Furthermore, blood from 10 healthy volunteers was collected for the analysis of the different Micra material components. A total of 9 ml venous blood was collected from each volunteer and each condition using blood collection tubes for heparin plasma. The study protocol was performed in accordance with the Declaration of Helsinki and was approved by the local ethics committee. All volunteers provided written informed consent to participate in the study before enrolment.

Micra pacemakers and 1 g gold, steel, titanium, tungsten and nitinol wires were incubated for 48 h at 37 °C and 5% CO_2 in 9 ml of heparinized whole blood. To avoid undesired influences from bacterial contaminations, Micra pacemakers and wires were previously disinfected using 70% ethanol and were exposed to UV radiation for 15 min. The incubation step was performed in 25 cm^2 cell culture flasks with filter screw caps to enable gas exchange. After 48 h blood was collected from each

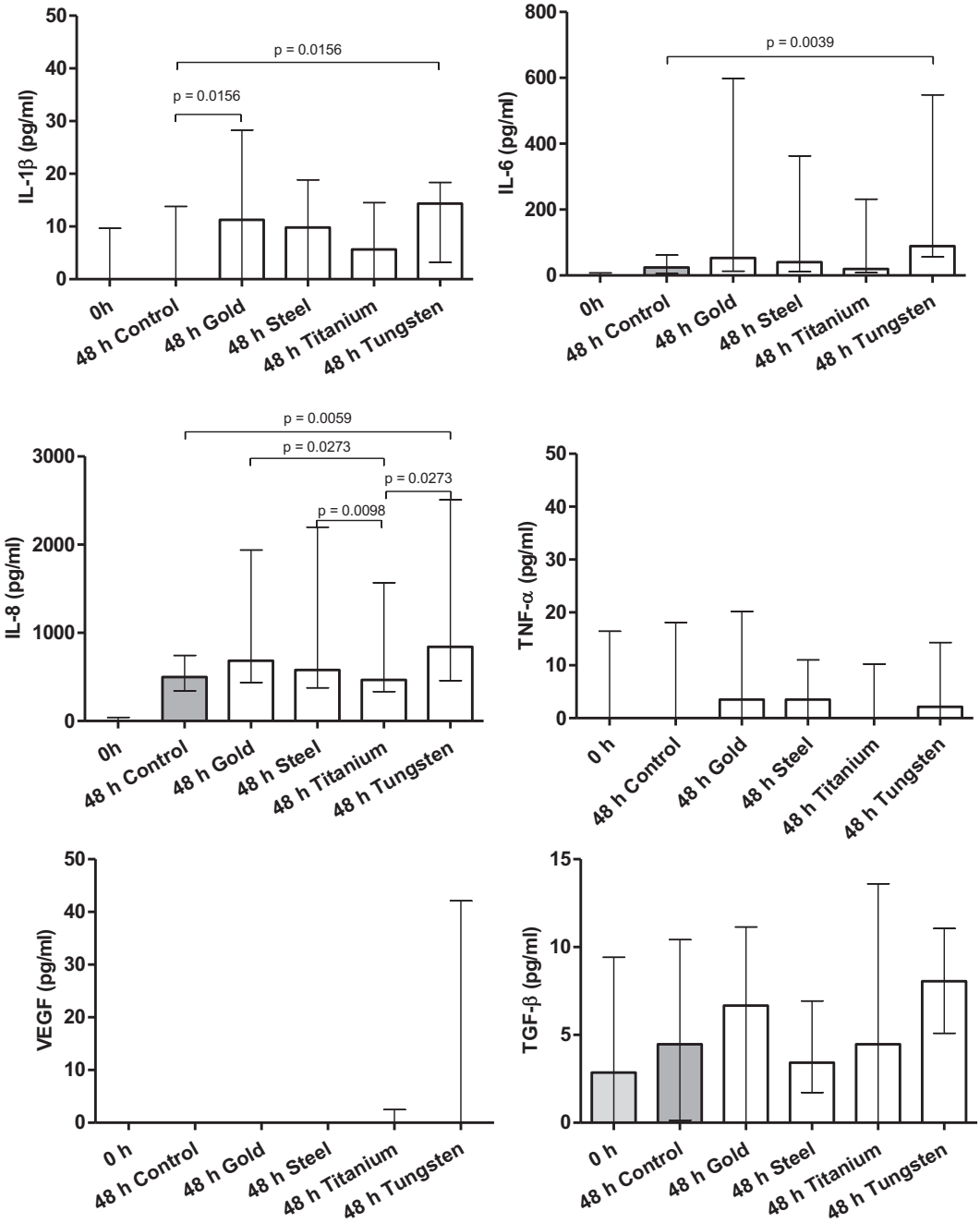

Fig. 2. Analysis of the effect of single wires on cytokines release in heparinized whole blood. Exposure to gold led to a significant increase in IL-1β concentration, Exposure to tungsten led to a significant increase in IL-1β, IL-6 and IL-8 compared to controls.

flask and centrifuged at 3200 rpm and 4 °C for 15 min. Supernatant was then stored at −80 °C until further analysis. In addition to that, one sample of 9 ml heparin plasma was centrifuged and frozen at time-point zero; another heparinized whole blood sample was incubated for 48 h at 37 °C and 5% CO_2 as well, although without Micra material components, serving as negative control.

Analysis of inflammatory cytokines and chemokines

Plasma levels of inflammatory cytokines such as IL-1β, IL-6 and TNF-α and chemokines like IL-8 were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN). The levels of TGF-β1 and VEGF were analysed by adequate ELISA kits as well. All ELISA procedures were performed according to the supplied manufacturer's instructions.

Plasma samples and standard proteins were transferred to the wells of a microplate, which was previously labelled with an adequate capture antibody. Samples and standards were incubated for 2 h at room temperature. Afterwards, plates were washed and a biotin-labelled detection secondary antibody was added to each well. Plates were again incubated for 2 h. Following another washing step, streptavidin-horseradish-peroxidase was added. After 20 min incubation and a final washing step tetramethylbenzidine (TMB; Sigma-Aldrich, St. Louis, MO) was added leading to a blue colour reaction. Reaction was stopped and protein concentration was measured at 450 nm with a Microplate reader (Bio-Rad Laboratories, Vienna, Austria).

Statistical analysis

Graph Prism software (GraphPad-Software, La Jolla, CA, USA) was used for all statistical analyses. To evaluate significant differences between the groups, the Wilcoxon matched pairs test was used. Medians and interquartile ranges are shown in graphs. A $p < 0.05$ was considered to be of statistical significance.

Results

Examination of effects of the whole Micra pacemaker system in vitro on inflammatory cytokines

All devices used in this project were original Micra pacemakers with full functionality.

As our first experimental test, we incubated the whole Micra system in heparinized whole blood samples. Fig. 1 gives a detailed overview on cytokine reactions after in vitro exposition to the whole Micra device. When exposed to the Micra device, IL-6 significantly increased (5.2 pg/ml to 33.1 pg/ml; $p = 0.02$) and IL-8 significantly decreased (839 pg/ml to 502 pg/ml; $p = 0.0124$) compared to controls, while TNF-α and VEGF did not increase at all. Additional findings were a slight increase in IL-1β and a slight decrease in TGF-β levels, however, both were not of statistical significance.

Examination of single Micra components

In a second step, we sought to analyse the effect of single wires, of every single metal component used in pacemaker devices. Exposure to gold led to a significant increase in IL-1β concentration (0 pg/ml to 11.3 pg/ml; $p = 0.0156$). As shown in Fig. 2, the exposure to tungsten led to an even more obvious increase, affecting levels of IL-1β (0 pg/ml to 14.4 pg/ml; $p = 0.0156$), IL-6 (24.6 pg/ml to 88.9 pg/ml; $p = 0.0039$) and IL-8 (497 pg/ml to 842 pg/ml; $p = 0.0059$) significantly compared to controls.

In a separate experiment session we tested nitinol wires on its immunogenic effects. A significant increase in IL-1β (8.8 pg/ml to 16.3 pg/ml; $p = 0.0391$) and IL-8 (872 pg/ml to 1317 pg/ml; $p = 0.0234$) compared to control was observed (see Fig. 3).

Discussion

Advances in technology lead to the dawn of a complete new era of pacemaker treatment. Since a total endovascular device placement is a completely new therapeutic option, long-term effects either at the implantation site or as a systemic reaction are currently not well known. First clinical data from autopsies showed unexpected complete or partial encapsulation with histological evidence of inflammation. The concept of this in vitro study was to study levels of typical inflammatory cytokines when heparinized whole blood was exposed to the Micra pacemaker device. Moreover, our aim was to find out which particular metallic component might be responsible for these changes. Therefore, typical metal components of pacemaker devices were analysed one by one.

Gold and its nanoparticles are considered to be non-immunogenic and non-cytotoxic [10]. Insignificant elevations of cytokine levels in IL-6, TNF-α, IL-1β and MCP-1 (mononuclear chemotactic protein 1) could be found in the past [11]. Titanium is known to be well tolerated in humans and therefore it is a widely used material for implants and alloys [12]. In patients carrying an orthopaedic implant, significant increases in cytokine concentration of TNF-α, IL-1β and IL-6 could be documented, without any evidence of toxicity [13,14]. Steel is known for having higher toxic effects than other common materials [15], a significant decrease in monocyte and macrophage survival rate could be observed in the past [16]. Furthermore, a reduction of leucocyte migration to the implant site could be shown in prosthetic implants.

Tungsten, a very frequently used material in medical is considered to be of well toleration. Despite a marginal elevation in serum levels [17], no cytotoxic effects are expected in the case of regular corroding rates of the implanted material. More recent papers suggest a potential toxicity in long-term use [18].

Nitinol, the material used in the anchor tines of the Micra, is a 55:45 Nickel/Titanium alloy. Due to its extraordinary elasticity and its thermal shape memory effects [19], it is a popular component in many medical devices. Immunologic effects of nitinol are considered to be comparable to stainless steel, while contribution to toxicity could not be observed [20,21]. After exposure to the whole Micra system, we could observe a significant increase in IL-1 β, while IL-8 showed significant decrease.

Since all of these markers are well known for their contribution in inflammatory pathways, the major question is, whether the achieved concentrations are high enough to be of clinical relevance. Several authors reported a mean in vitro control level of IL-1β in blood plasma from 0.0 pg/ml to 14.7 pg/ml [22–27], which matches our obtained results. In summary, our findings support the current theory, based on clinical data, of a local subclinical response, without having any systemic relevance.

Kypta et al. published a case of a fully encapsulated device, which could found in an autopsy [9,28]. Interestingly, the pathohistological analysis showed coverage by layers of lymphocytes and fibrous tissue. Additional immunochemistry gave evidence for an ongoing chronic inflammation. Tjong el al. observed a fibrous capsule containing plenty of myofibroblasts, but no coverage of the device by endothelial cells in the case of an 81 years old male [8]. However, encapsulation itself is an expected and appreciated process in other intracardiac devices, such as occluder systems, which reduces the risk of device infection.

Therefore, one may speculate, these unexpected findings of encapsulation might even be of protective value.

After all, these processes do not seem to be a major challenge in clinical treatment, since all out of the currently published clinical cases did not show any kind of device malfunction. Even the capture thresholds remained stable despite encapsulation. Encapsulation processes might be of clinical relevance in case of device malfunction or infection, when a removal has to be considered. In case of an overwhelming

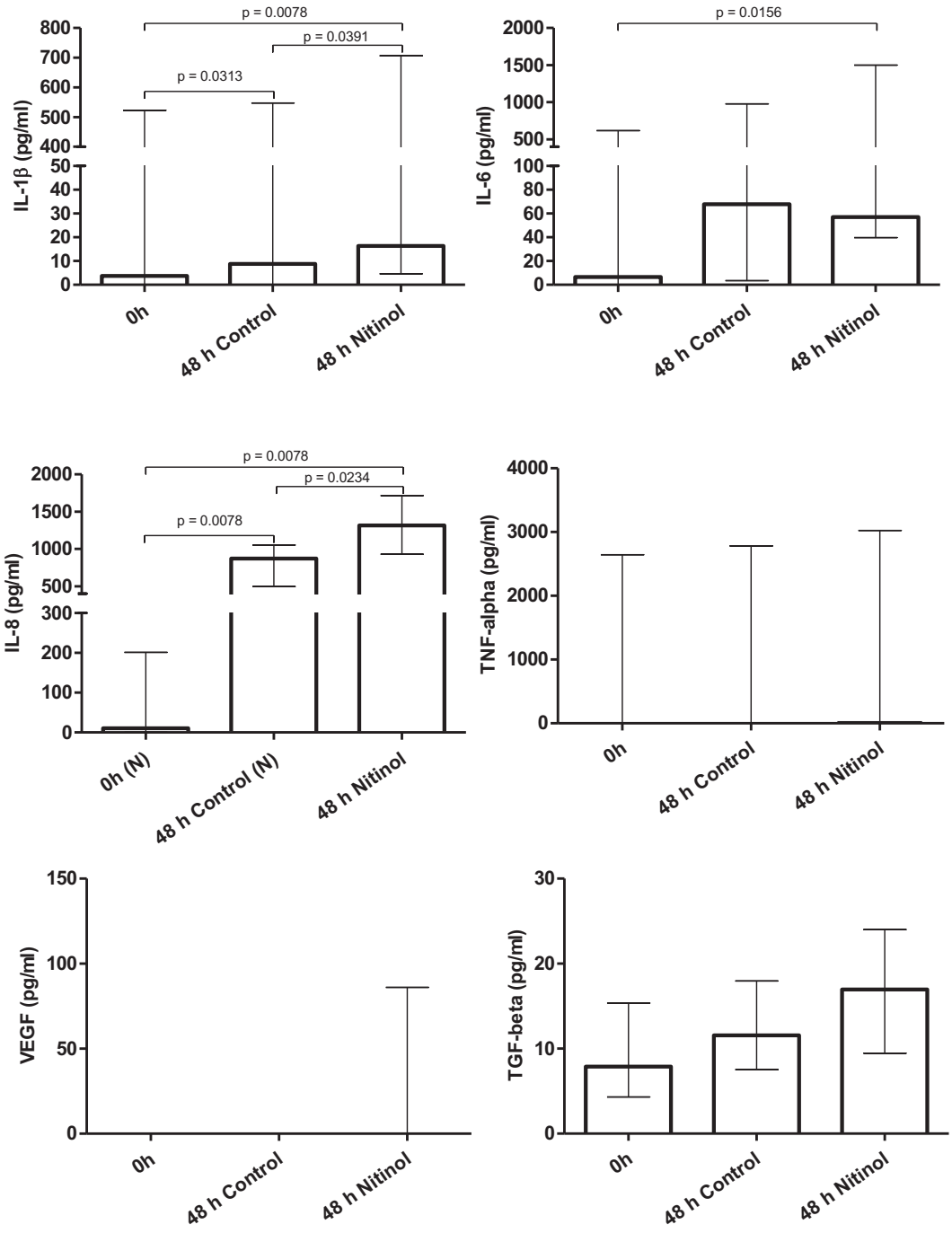

Fig. 3. Effects of nitinol wires on cytokines levels in heparinized whole blood: a significant increase in IL-1β and IL-8 compared to control was observed.

encapsulation, the placement of an additional device might be challenging and the impact on device performance remains unclear.

Another major aspect to deal with in the clinical setting is the intake of drugs. Medication with a potential effect on encapsulation could include anticoagulants with well-known anti-inflammatory effects. Further studies are needed, to investigate cytokine changes and local inflammatory mechanisms in an in vivo setting.

Within our results on the whole micra device, we could observe a significant increase in IL-6 concentration.

A temporary increase of IL-6 levels in vivo could eventually be explained by the endothelial trauma caused by the anchor system, since IL-6 is well known to be a key player in the regulation of acute phase protein synthesis.

Otherwise IL-6 contributes in the shifting process from acute to chronic inflammation. These shiftings, which are mainly driven by monocyte recruitment, might eventually lead to a persisting elevation of IL-6 concentrations in long-term treatment.

Moreover, we hypothesize, the effects of IL-6 on fibroblasts and endothelial tissue could serve as a possible explanation for the observed encapsulations.

Limitations

As a major limitation however, levels of inflammatory cytokines in vitro cannot be compared to blood plasma levels in vivo. Cytokine expression can potentially be induced by blood withdrawal, processing

and incubation itself, which might result in a mismatch of in vitro and in vivo results. Furthermore, all blood samples were taken from young healthy individuals without any relevant drug intake, which does not exactly reflect the circumstances, which are encountered in a pacemaker cohort.

Moreover, the surfaces of Micra pacemakers in the original packaging are coated with parylene. Given the fact that all devices were former in clinical use and had to undergo meticulous washing before the start of our experiments, we cannot completely exclude any damage to this parylene layer.

Conclusion

For the first time, we could observe an immune response to the Micra leadless device as well as in common pacemaker components in our in vitro model. Analysis of single components showed the highest alterations of cytokines when blood was co-incubated with gold and tungsten. Given the fact, that the analysed cytokines are known for their immunogenic effects, our study supports the theory of a subclinical and local inflammation without systemic relevance. However, further studies in an in vivo setting are needed, to validate clinical relevance and to consider possible therapeutic consequences.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgements

This manuscript is dedicated to the memory of our colleague, co-author and friend Dr. Alexander Kypta, who had the initial idea for this study. His innovative ideas, his scientific skills and his clinical experience will be missed.

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