

In vivo monitoring of gut inflammation in the insect larvae of *Manduca sexta* by ¹⁹F magnetic resonance imaging

Anton G. Windfelder

Fraunhofer IME , Department Bioresources, Ohlebergsweg 12, 35392 Giessen, Germany
Justus Liebig University Giessen, Clinic for Diagnostic and Interventional Radiology, Klinikstraße 33, 35392
Giessen, Germany

Mammalian model organisms are increasingly subjected to ethical concerns and restrictions (3R Principle). Therefore, we have established the insect larva of *Manduca sexta* as a new alternative model organism for the ¹⁹F-based detection of inflammation in magnetic resonance imaging.

We used bacteria-induced gut inflammation as a positive control to demonstrate the feasibility of ¹⁹F-MRI in insects using nanoemulsions of perfluorocarbons (PFCs) at 9.4 T. Simultaneous acquisition of morphologically matching fluorine (¹⁹F) and proton (¹H) images enabled an anatomic localization of PFCs after application in the anterior and posterior midgut region of the insect larvae. Afterward, an ex vivo examination of the insect's midguts confirmed inflammatory lesions in the anterior and posterior midgut regions with severe melanizations. In independent experiments using flow cytometry and rhodamine-labeled PFCs, we determined plasmatocytes and granulocytes, the two main fractions of *Manduca sexta* hemocytes ("White Blood cells" of insects), as a sink for injected PFC nanoparticles. Further, plasmatocytes and granulocytes showed significant percentual alteration from non-inflamed control animals, confirming the positive control's inflammatory status.

Since innate immunity is highly conserved between insects and mammals, insect larvae are suitable as alternative 3R-compatible in vivo animal models for screening new contrast agents, protocols, and imaging modalities. Also, this imaging platform may allow the sublethal detection of insect inflammation as a persuasive new tool in ecotoxicology.