Resolvin E1 heals injured cardiomyocytes: Therapeutic implications and H-FABP as a readout for cardiovascular disease & systemic inflammation

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Abstract

Acute coronary syndrome (ACS) is the leading cause of death and disability in the US, and SARS-CoV-2 COVID-19 the third leading cause of death. As reported extensively, many COVID patients admitted to BUSM with severe pulmonary pathologies also had inflammatory cardiovascular (CV) pathologies that increase the likelihood of major adverse cardiovascular events (MACE) during hospitalization and often after discharge. Rapid and reliable detection of COVID is crucial, since MI can occur as early as 2 days after admission. Detection and therapy will be enhanced by our mechanistic studies of heart fatty acid binding protein (H-FABP). Our study is based on the hypothesis that cardiovascular events and inflammation will be detected by leakage of H-FABP, after cardiomyocyte membrane damage. H- FABP is a small soluble intracellular molecule found almost exclusively in the cardiomyocyte cytoplasm has been used as a clinical measure of damage in patients with heart failure and myocardial infarction (MI). It has been shown to be a sensitive, rapid, and specific assay for detecting CVD quickly upon admission. Our in vitro cell studies demonstrated that H-FAB measured by ELISA is a sensitive and reliable biomarker of cardiomyocyte damage induced by lipopolysaccharide (LPS), and healing of the membrane by RvE1,a specialized pro-resolving mediator (SPM) derived from the Omega-3 fatty acid, eicosapentaenoic acid (EPA), a dietary nutrient that balances inflammation to restore homeostasis. EPA has also been shown to promote muscle repair after MI.

Introduction

H-FABP is a low molecular weight (15 kDa) protein (**Fig.1)** solubilized in the cytoplasm of cardiomyocytes and functions to transport free fatty acids from the plasma membrane to the mitochondria to facilitate fatty acid metabolism (**1, 2, 3**). H-FABP is not attached to an intracellular protein and can quickly leak out of cardiomyocytes following myocardial cell death or injury.

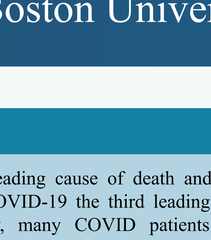


Figure 1. 3D structure of FABP, illustrating the general backbone structure ("beta- clamshell") of all FABPs with an entrapped fatty acid inside determined in the Hamilton Lab.

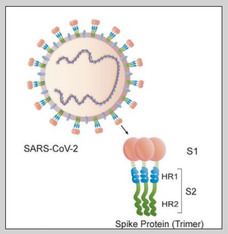


Figure 2: Structure of SARS-CoV-2 spike protein

It has been shown that SARS-CoV-2 spike protein (**Fig. 2**), which is highly infectious, binds to LPS and boosts proinflammatory activity. The spike protein is bound to the SARS-CoV-2 virus membrane and desorbs rapidly, which facilitates systemic viral infection, We will perform parallel trials of damage and healing in cardiomyocytes with purified spike protein, which does not require the restrictions a BL3 lab for studies of the SARS-CoV-2 viral particles.

Methodology

Using commercial ELISA sandwich assays, we tested our hypothesis that H-FABP will be detected in the supernatant of cells after cardiomyocyte membrane damage and in the blood of patients with COVID and/or other inflammatory comorbidities.

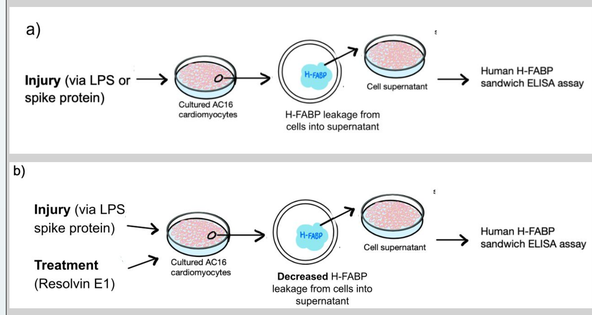


Figure 3: Injury to cardiomyocytes and H-FABP leakage before **(a)** and after **(b)** treatment of Resolvin E1.

Blood samples were diluted 1:15 in sample diluent provided by the commercial human H-FABP ELISA kit. To induce cardiomyocyte cell membrane damage we applied the Endotoxin lipopolysaccharide (LPS) in cultured immortalized cardiomyocytes and stem cell-derived cardiac cell systems, to induce cell membrane damage and inflammation. ELISA assays showed instantaneous concentration-dependent leakage of H-FABP into the buffer of cultured cardiomyocytes, and H-FABP was significantly decreased in cells treated by resolvins. The assay was processed through a microplate reader at 450 nm to obtain the quantitative value entered in our graphs and tables. After establishing the reproducibility of the high sensitivity of H-FABP, we tested our hypothesis that inflammation-lowering therapeutics (RvE1) will reduce the damage by LPS to heart cells and that can be validated by decreased leakage of H-FABP by the ELiSA methods in 12-well plates. For quantification of H-FABP, supernatant samples were centrifuged after collection and diluted 1:10 in sample diluent provided by the ELISA kit. We obtained blood samples from the BMC COVID biorepository, which collects blood from patients upon admission to the hospital. This biorepository has clinical information, such as demographics, disease severity, treatments, complications, and comorbidities (such as End Stage Renal Disease).

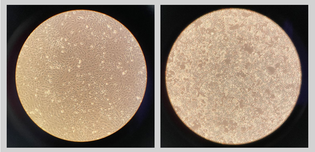


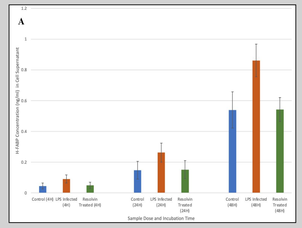
Figure 4: Cultured AC16 human cardiomyocytes before (left) and after (right) incubation with 1ng/mL LPS for 24 hours. Cell nuclei can be seen as the bright spots. We found greater H-FABP content in the supernatant from the cells with LPS exposure.

Our studies support a unifying mechanistic hypothesis of high systemic inflammation and therapy based on inflammation resolution that can be achieved by lifestyle changes to supplement proven therapeutics for the acute treatment of infection. The cohort of the study will benefit from inexpensive therapeutics and from a holistic approach.

Results

Overall, we found significant differences in H-FABP leakage comparing various diagnosed comorbidities. H-FABP leakage in blood from patients with and without COVID-19 infection showed smaller differences. Measurement of H-FABP encompasses different comorbidities and different extents of the comorbidities.

Figure 5: Concentrations of H-FABP measured in AC16 cell supernatant from LPS-exposed cardiomyocytes with RvE1 treatment after 4h (acute), 24h, and 48h (chronic) incubation times. All 12 wells were incubated for either 4 hours, 24 hours, or 48 hours, and 1 ml supernatant samples were collected after each time period had elapsed.



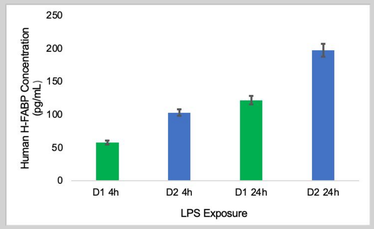


Figure 6: H-FABP concentrations with different LPS doses (low dose: **D1**, ***5 µg/ml [green bars] and high dose:* D2, *25 µg/ml [blue bars])** and incubation times of 4 or 24 hours.

Figure 7: H-FABP in blood of patients admitted to BMC with respiratory symptoms and with clinically diagnosed comorbidities of end stage renal disease (ESRD), cardiovascular disease (CAD), and diabetes. The tables below show patients who were diagnosed with COVID (bottom) and "controls" without COVID (top).

COMORBIDITY COMPARISON AMONG BLOOD SAMPLES FROM COVID NEGATIVE PATIENTS

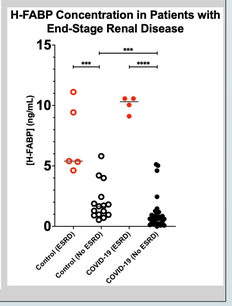
Patient	CAD	ESRD	Diabetes	No comorbidities	H-FABP concentration
1		+	+		5.40
2	+	+			5.34
3		+			4.63
4	+	+	+		11.12
5	+	+	+		9.43
6	+				4.21
7	+		+		4.00
8	+				5.81
9	+				7.34
10	+	+			3.10
11				+	1.29
12				+	1.72
13				+	1.88
14				+	1.26
15				+	0.96
16				+	1.66
17				+	0.71
18				+	0.98
19				+	0.91
20				+	0.54

COMORBIDITY COMPARISON AMONG BLOOD SAMPLES FROM COVID POSITIVE PATIENTS

Patient	CAD	ESRD	Diabetes	No comorbidities	H-FABP concentration
21			+		4.608
22			+		2.451
23		+			9.108
24	+	+	+		10.05
25		+	+		10.57
26		+			9.11
27	+	+			10.57
28			+		5.03
29			+		4.61
30				+	1.171
31				+	0.624
32				+	0.608
33				+	0.12
34				+	0.69
35				+	0.30
36				+	0.22
37				+	0.76
38				+	0.38
39				+	0.16
40				+	0.08

Patients in both groups with 2 or more comorbidities (CAD, ESRD, diabetes) have higher blood H-FABP than those with 1 or no known comorbidities (green). Patients with no known comorbidities had H-FABP levels less than 2.

Figure 8: Higher H-FABP concentrations observed in blood from patients with ESRD regardless of whether they had COVID-19.



Conclusion

Dose-dependent and time- dependent amounts of H-FABP released from cardiomyocytes into the media supernatant were measured by ELISA. Increased LPS exposure resulted in an increased H-FABP concentration ($p < 0.05$, *Lipopolysaccharide was used in cultured cells as a model for inflammation damage to optimize* standard error = 0.071, $N = 33$), showing that heart muscle damage can originate at a cellular level. H-FABP was detected at low concentrations (ng/ml). The addition of RvE1 caused *decreased* leakage of H-FABP, which validates our hypothesis of inflammation as a mechanism of injury. The treatment with RVE1 was dose- and time-dependent With a 4 hour incubation, H-FABP concentration decreased by 72.3%. With a 24 hour incubation, H-FABP concentration decreased by 99.4%.

Since the early studies linking CVD to other risk factors, SARS-CoV-2 infection has been documented to increase inflammation of the heart muscle in up to 60% of patients recovering from COVID-19. As the authors[1] conclude, there still is not a clear link between risk factors and long term problems. There is increasing evidence that long term SARS-CoV-2 infection greatly increases the risk of long-term kidney disease. Our proposed H-FABP measurements in patient blood with identified inflammatory-driven comorbidities emphasize the unique value of H-FABP in monitoring comorbidities with multiple risk factors, including ESRD, CVD), and diabetes. A major gap in the healthcare of patients is the need for continuous attention for cardiac injury and whether comorbidities improve with timely treatment, which will be enhanced by our therapy with RvE1 and other SPMs to prevent further cardiac issues. Early identification, diagnosis, and treatment decrease the risk of further downstream adverse health consequences. Our study promotes and provides a basis for the standardization of criteria with highly validated blood biomarkers that encompass heart, vascular, and other organ pathologies that will enable informed decision-making by physicians.

Future Directions

We will perform parallel trials of damage and healing in cardiomyocytes with purified spike protein, which does not require the restrictions a BL3 lab for studies of the SARS-CoV-2 viral particles. The importance of spikes is increasingly relevant because of the new COVID spike vaccine. It is important to extend our spike studies to understand the mechanism of leakage and damage by spikes alone. We have preliminary data showing that spike damage to cells is on a similar scale to LPS.

Acknowledgements

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