The role of Neutrophil Gelatinase Associated Lipocalin in linking Depression and Heart Failure.

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Introduction

Depression is a well-known co-morbidity in heart failure. In fact, approximately half of all patients with heart failure suffer from signs of depression. Co-morbid depression severely worsens prognosis in these patients. However, the majority of these patients remain untreated; beside the point that the cardiologist regards depression often as a "natural" response on a life threatening condition, a rational for adequate therapy is hampered by a lack of knowledge concerning the underlying mechanism of this neurocardiac interaction. Moreover, optimal cardiovascular treatment is not associated with lower incidence of depression, while the other way around, successful anti-depressant therapy is not associated with improved prognosis. These observations may suggest a common mechanism, rather than a direct causal relationship underlying heart failure and depression.

Inflammation may be an underlying biological mechanism and a common denominator in heart failure as well as depression. Heart failure and depression share increased circulating levels of pro-inflammatory cytokines, including TNFα, while co-morbidity is associated with higher levels than each condition alone. We hypothesize that an initial functional immune response on local tissue damage, is not able to keep this process local, either due to repeated exposure or due to an exaggerated inflammatory response (reviewed by Quan, 2014(1)). Hence, immunosensitive afferent nerves can be stimulated, as well as inflammatory factors may enter the circulation, both of which may enter the brain either directly or via the blood-brain-barrier, and subsequently induce neuroinflammation. Neuroinflammation could provide protection to limit CNS damage, but may confer long-term consequences related to chronic exposure to inflammation associated neurodegeneration. The localization of these processes in the brain then determines the specific behavioral output. This hypothesis is presented for myocardial infarction in figure 1.

Studies in rats with myocardial infarction support a role for TNFα in neuroinflammation with depression and heart failure. Myocardial infarction evokes elevated levels of cardiac as well as circulating TNFα, starting 30 minutes after MI, and substantially further increases the following weeks. We previously showed that myocardial infarction induces focal leakage of the blood-brain-barrier at least up to 3 days post-infarct; an effect that can be mimicked by peripheral TNFα infusion. In the brain, amongst others, TNFα expression is increased at mRNA level as well as protein level in the hypothalamus, but not the cortex. In the paraventricular nucleus of the hypothalamus, microglia activation is observed up to 16 weeks after myocardial infarction. Moreover, in these rats, signs of depressive behavior are reported, that can be inhibited by TNFα blockade (etanercept) (2). However, while the hypothalamus can be regarded as a relative non-specific brain area in relation to more complex behavioral changes as depression, TNFα can be regarded as an important, though relative non-specific cytokine, as it is activated in numerous conditions. Therefore the aim of our research is to further unravel the underlying mechanisms involved in the heart failure-(neuro)inflammation-depression triangle.

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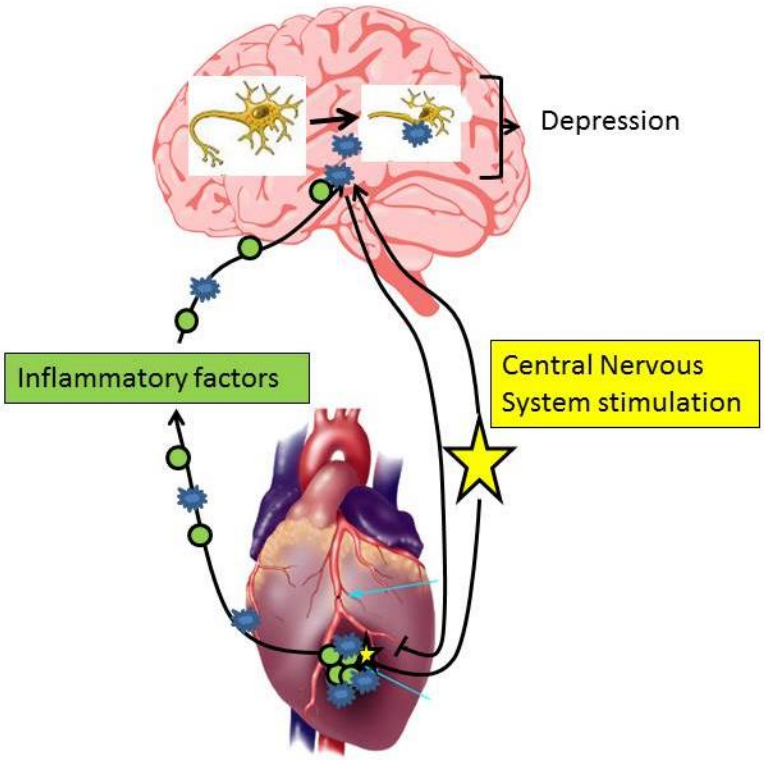

Figure 1: Hypothesis concerning the role of inflammation in the development of heart failure and depression. A local inflammatory response on tissue damage due to myocardial infarction evokes activation of inflammatory cells and factors to "heal" the infarct. This initially local inflammatory response can be expanded into the brain either by stimulation of afferent nerves, or by entering the circulation and crossing the blood brain barrier. Localized inflammatory responses in the brain may then be associated with depressive behavior.

A detailed experiment investigating novel targets induced by TNFα, revealed Neutrophil Gelatinase Associated Lipocalin (NGAL) as an interesting candidate. It can be produced by neurons, microglia and astrocytes, and is activated throughout the brain at peripherally induced inflammation by LPS. Moreover, as NGAL is a well-recognized predictive factor for prognosis in heart failure, it is also found increased in late life depression (3). Most recently we showed a significant association of depressive symptoms in patients with heart failure and increased plasma NGAL levels, even after correction for cardiac- and renal dysfunction(4). This association, however, was observed for the somatic, rather than the cognitive aspects of depression, indicating a specific subset of elements of depression to be affected.

In the present study we investigated 3 elements of depression after myocardial infarction in rats, and evaluated a potential role of NGAL.

Methods

Experimental myocardial infarction in rats.
Male Wistar rats were subjected to experimental myocardial infarction by surgical ligation of the left descending coronary artery or sham surgery. Ligation results in a massive transmural infarct in the free wall of the left ventricle leaving papillary muscles largely unaffected, hence leading to pump failure rather than cardiac arrhythmias.

Behavioral tests

Previously, we showed that rats with myocardial infarction showed depressive-like behavior, presented as decreased interest in their environment, measured as lower time spent on exploratory behavior in an open field test, and lower social interest, measured as time spent on social interaction (5). Therefore, 3 weeks after myocardial infarction or sham surgery, when irreversibly damaged cardiac tissue is replaced by scar tissue, behavioral testing was performed. "Depressive-like" behavior was evaluated from exploration in the open field, as measures for interest in environment; latency to leave the home cage to enter a novel area, as measure of motivation; and a sucrose preference test, as measure for loss of pleasure; anhedonia. All behavioral tests were performed during the dark phase; the active period of the rat.

For the open field test: rats were placed in the center of the open field (1m x1m) and were allowed to explore it for 5 minutes. Behavior was recorded and a total review of the rats behavior was obtained off-line using E-line software. Time spent on the different behavioral elements; sniffing, walking, rearing, exploring wall, resting, grooming, other, was calculated and time spent on exploration was regarded the summation of the first 4 elements. Less time spent on exploration is regarded as lower interest in environment; a sign of depression.

Free exploration: in this test we gave the rat a choice to whether or not leave its home cage and explore the open field. For that the home cage with the rat in it was placed in the open field, the lit was removed, and the rats was allowed for 5 minutes to climb out and explore the open field. The latency to leave the home cage was recorded and a higher latency is regarded as sign of depression; a lower motivation to explore a new environment (5).

Anhedonia was obtained from a sucrose preference test. For that, after a habituation session of 2 bottles and the taste of sucrose, rats were subjected to the choice of normal water or 1% sucrose for the first 5 hours of the dark period (period in which rats drink most). A lower percentage of sucrose of the water intake is regarded as sign of depression.

Although the used behavioral tests, except for the free exploration, may be used rather generally, this combined setting may provide a novel and relevant way to study the mechanism underlying "depression", which clinically is a highly variable combination of symptoms.

Tissue and plasma analyses

Five weeks after myocardial infarction or sham surgery, rats were sacrificed. Plasma and cerebrospinal fluid samples (CSF) were collected for measurement of NGAL levels. Subsequently the brain was perfused with formaldehyde for fixation and processed for immunohistochemical staining of NGAL. The heart was dissected and processed for measurement of infarct size as the % of left ventricular circumference at mid-ventricular level.

Experimental procedures were approved by the local animal experiments and welfare committee of the University of Groningen, the Netherlands.

Results

In the present study, rats with myocardial infarction did not show statistically significant less exploration in the open field test. Latency to leave the home cage appeared higher in rats with myocardial infarction (74±12 versus 58±9 sec). Difference in sucrose preference, 77±6% in infarcted versus 83±4% in sham rats, did however not reach statistical significance. Time spent on exploration in the open field was not correlated to either latency to leave the home cage or sucrose preference. Sucrose preference and latency to leave the home cage were significantly correlated ($p=0.024$). However, this correlation was found positive, leaving the rats with highest sucrose preference displaying the longest latency.

Plasma levels, as well as CSF levels of NGAL, were higher in infarcted compared to sham rats (plasma: 170±37 versus 115± 25 ng/ml; CSF 25.9±17.2 versus 6.5±2.9 ng/ml), but did not reach statistical significance. The number of NGAL positive cells in the magnocellular part of the paraventricular nucleus of the hypothalamus (PVN), but not in the parvocellular part, was significantly increased after myocardial infarction (45±7 versus 27±2). No differences were observed in the different areas of the hippocampus, nor in the prefrontal cortex.

Interestingly, whereas plasma levels of NGAL were significantly correlated to infarct size, but not to brain NGAL expression, NGAL expression in the magnocellular part of the PVN appeared significantly correlated to exploration in the open field, while correlations with latency (p=0.100) and sucrose preference (p=0.053) did not reach statistical significance, but showed a tendency. Moreover, magnocellular expression of NGAL was significantly correlated to the number of microglia in the PVN (p=0.023).

Discussion/conclusion

In the present study, 3 different elements of depressive-like behavior were evaluated in a rat model for myocardial-infarction associated depression; loss of interest in environment, lack of motivation and anhedonia. Not all measured elements of depressive-like behavior did statistical significantly differ between sham and infarcted rats, though a clear tendency was present. Since within the same animals, scores of these elements were either not correlated, or correlation was even in the opposite direction, the elements may indeed reflect different (and none-related) symptoms of depression. Since different, and even opposing effects are also observed in patients with depression (DSM-V), combining different elements of depression using different (non-aversive) tests in the same animal, may provide a more relevant approach to investigate the complex syndrome of depression in animal models, than the common single-test studies.

Experimental myocardial infarction in rats increases production of NGAL, resulting in increased plasma levels, related to infarct size. This indicates that plasma NGAL levels may reflect severity of cardiac dysfunction. However, since no correlation between plasma levels of NGAL with brain expression of NGAL was observed, nor with any measured aspect of depressive behavior, plasma NGAL may not directly affect brain functioning. Expression of NGAL in the brain was significantly increased in the PVN, which correlated to exploration behavior in the open field while a tendency towards correlation with the other depression parameters were observed. This may indicate that increased brain NGAL expression may reflect ”depressive like behavior” associated with heart failure rather than heart failure itself.

Both circulating and brain-associated NGAL may reflect different aspects of heart failure that are important for prognosis. The lack of consistent associations between circulating and brain NGAL may support the idea of a common underlying mechanism, rather than a causal relationship for heart failure and co-morbid depression. Since NGAL may play an important role in inflammatory processes, the study may provide evidence for our neuroinflammation hypothesis.

References

1. Quan, N. (2014). In-Depth Conversation: Spectrum and Kinetics of Neuroimmune Afferent Pathways. *Brain, Behavior, and Immunity*.(in press)

2. Grippo, A. J., J. Francis, R. M. Weiss, R. B. Felder, and A. K. Johnson. (2003). Cytokine Mediation of Experimental Heart Failure-Induced Anhedonia. *American Journal of Physiology.Regulatory, Integrative and Comparative Physiology* **284** (3): R666-73.

3. Naude, P. J., U. L. Eisel, H. C. Comijs, N. A. Groenewold, P. P. De Deyn, F. J. Bosker, P. G. Luiten, J. A. den Boer, and R. C. Oude Voshaar. (2013). Neutrophil Gelatinase-Associated Lipocalin: A Novel Inflammatory Marker Associated with Late-Life Depression. *Journal of Psychosomatic Research* **75** (5): 444-450.

4. Naude, P. J., P. M. Mommersteeg, W. P. Zijlstra, L. Gouweleeuw, N. Kupper, U. L. Eisel, W. J. Kop, and R. G. Schoemaker. (2014). Neutrophil Gelatinase-Associated Lipocalin and Depression in Patients with Chronic Heart Failure. *Brain, Behavior, and Immunity*. (in press)

5. Schoemaker, R. G. and J. F. Smits. (1994). Behavioral Changes Following Chronic Myocardial Infarction in Rats. *Physiology & Behavior* **56**: 585-589.