Antichemokine treatments in acute ischaemic cardiovascular diseases

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Summary

Inflammatory processes have been shown to be major pathophysiological determinants of patient vulnerability for acute ischaemic cardiovascular diseases. Among soluble inflammatory mediators, chemokines have been investigated as potential proatherosclerotic factors in both humans and animal models. In particular, several chemokines were shown to be related to plaque vulnerability and to predict independently the risk of ischaemic events. Moreover, chemokines are under investigation in secondary prevention. Considering the pathophysiological relevance of chemokines in atherogenesis, the development of therapeutic compounds selectively targeting their bioactivities might represent a promising approach to the prevention of both plaque rupture and adverse evolution of ischaemic injury. Although several compounds have been investigated in animal models with some promising results, at present there is no experimental evidence for the use of antichemokine mediators in clinics. Some potential safety concerns (immunosuppression and allergic reactions) have been indicated as potential limitations. The aim of this narrative review is to provide an update of the role of chemokines as biomarkers and promising therapeutic tools in acute ischaemic cardiovascular diseases.

Key words: chemokines; ischaemia/reperfusion injury; acute myocardial infarction; ischaemic stroke

Introduction

Atherosclerosis is a progressive disease affecting nearly all individuals all over the world. Atherogenesis slowly progresses from childhood until forming advanced lesions in adulthood [1]. Advanced lesions may remain stable or evolve into rupture-prone plaques (also termed vulnerable plaques). This acute event is the leading cause of life-threatening ischaemic events, such as myocardial infarction and ischaemic stroke.

Since traditional risk factors, which emerged from Framingham heart study, failed to predict precisely the risk of plaque rupture, a new approach has been explored in the last decade [2]. Naghavi and co-workers proposed a novel paradigm of “vulnerable” patients focusing on three parameters (systemic, intraplaque and peripheral tissue vulnerabilities) and emphasising the role of inflammation as the driving force leading to “global” patient vulnerability [3]. Combined with the general concept of atherosclerosis as a systemic disease, this approach highlighted the importance of systemic biomarkers (including mediators of inflammation, prothrombotic fac-
tors and markers of matrix degradation) potentially to identify vulnerable plaques [4]. Many biomarkers have been investigated, but several studies are supporting a potential predictive role of chemokines for acute ischaemic events (table 1) [5–22]. Although circulating levels of several chemokines have been shown to predict future ischaemic cardiovascular (CV) events, their use as potential clinical biomarkers is still unvalidated.

**Chemokines in acute myocardial infarction and stroke**

Chemokines (chemotactic cytokines) are small heparin-binding proteins that regulate leucocyte trafficking to sites of inflammation. The systematic nomenclature and classification of currently known chemokines (almost 50) relies on the different spacing of two conserved cysteine residues at the N-terminus. The different spacing establishes chemokine quaternary structure, function and also their classification into four families: CC-, CXC-, CX3C- and XC-chemokines [23]. CC-chemokines attract mainly mononuclear cells to inflammatory sites. CXC-chemokines recruit primarily polymorphonuclear leukocytes to sites of acute inflammation. CX3C/1/fractalkine is the only member of CX3C family; XCL1/lymphotactin and XCL2/SCL-1β are members of the XC family [23].

Chemokine intracellular signalling is transduced by binding to specific G-protein-coupled seven-transmembrane receptors (a superfamily of 20 members) categorised on the basis of their specificity for chemokine family (CCR and CXCR) [23]. Since several chemokines bind to multiple receptors and vice versa, different combinations of chemokine and chemokine receptor expression are available on the cell surface, thus enabling “tailor-made” cell recruitment. In addition, although certain chemokines are constitutively expressed, others are inducible and up-regulated by environmental stimuli, further enhancing leucocyte recruitment [24].

In the ischaemic myocardium, several overlapping pathways might up-regulate chemokine expression, including oxidative stress, cytokines, the complement cascade, toll-like receptor and NF-κB [25]. However, chemokine bioactivities are not limited to neutrophil recruitment during the first inflammatory phase. For instance, the chemokines CXCL12 and CCL2 have been shown to protect cardiomyocytes directly [26, 27]. In addition, the chemokines CXCL12, CXCL1, CXCL2 and CCL2 were shown to induce angiogenesis and cell differentiation [28].

Also in the central nervous system, chemokines were shown to regulate both physiological and pathological processes. The CXCL12-CXCR4 axis might promote not only the inflammatory response [29], but also neural progenitor/stem-cell migration, proliferation and differentiation, both in neurogenesis [30] and after ischaemic stroke. We will discuss in the next paragraphs the specific role of CXC and CC chemokines in the pathophysiology of acute cardiovascular events.

**CXC chemokines**

CXC chemokines have been associated with both atherosclerotic plaque instability and ischaemia/reperfusion injury within heart and brain, owing to their potential attraction of neutrophils and monocytes [31]. In humans, CXCL8 is the prototype of the glutamate-leucine-arginine (ELR+) subfamily and the most investigated CXC chemokine. Its homolog chemokine in mice is CXCL2. Oxidised low density lipoproteins (oxLDL) strongly induced CXCL8 expression by monocytes [32]. In addition, CXCL8 has been shown to be released by other cells colonising atherosclerotic plaques, such as foam [33] and endothelial cells [34]. In experimental ischaemia/reperfusion injury, CXCL8 was detected in the border zone of the infarct [35], closely linked to neutrophil infiltration. Accordingly, treatment with recombinant CXCL8 [35] or anti-CXCL8 [36] antibodies enhances or prevents neutrophil infiltration, respectively. We recently suggested a direct role for CXCL8 in human carotid plaque vulnerability. In fact, patients with symptoms of ischaemic stroke had higher intraplaque levels of CXCL8 messenger ribonucleic acid (mRNA) as compared with asymptomatic subjects [37]. In addition, CXCL8 levels were also increased in serum [38] and cerebrospinal fluid [39] after an ischaemic stroke. CXCL1 was shown to enhance not only vascular inflammation [40], but also angiogenesis and endothelial progenitor cell (EPC) recruitment [41] together with CXCL8 [42], even if there is no agreement about their activities [43]. In the cerebrospinal fluid, CXCL1 levels positively correlate with the volume of cerebral hypodense areas (assessed with computed tomography [CT]), suggesting an involvement of this chemokine during early inflammatory phases after ischaemic stroke [44].

On the other hand, CXC chemokines lacking the ELR motif (such as CXCL9, CXCL10) have been shown to block the early healing phases after ischaemic injury. In addition, these chemokines have been described as are active angiostatic factors [45, 46] and inhibitors of fibroblast migration [47]. In contrast to other CXC chemokines, CXCL12 and its receptors CXCR4 and CXCR7 were clearly shown to induce beneficial effects. CXCL12 is expressed in atherosclerotic plaques [48] as well as in myocardium [49] and brain [50] after ischaemia, and it was shown to promote tissue recovery through EPC recruitment [51, 52]. It should be noted that modified CXCL12 may also have detrimental effects. In fact, the cleavage of CXCL12 by matrix metalloproteinase (MMP) 2 was shown to create a neurotoxic molecule that did not bind CXCR4, but had an increased affinity for CXCR3 [53].
<table>
<thead>
<tr>
<th>Chemokine</th>
<th>Author</th>
<th>Population</th>
<th>Study design (follow-up)</th>
<th>Outcome</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL2/MCP-1</td>
<td>De Lemos et al. [5]</td>
<td>2,270 from OPUS-TIMI 16 trial</td>
<td>Prospective observational (10 months)</td>
<td>Death and/or AMI</td>
<td>CCL2 was independent predictor of worst outcome (HR 1.42 [95% CI 1.02–1.98]; p = 0.04)</td>
</tr>
<tr>
<td></td>
<td>Kervinen et al. [6]</td>
<td>183 patients with chest pain (64 SA, 60 UA and 59 AMI)</td>
<td>Prospective observational (13 months)</td>
<td>CV events</td>
<td>Increased CCL2 was associated with increased risk (OR 1.85 [95% CI 1.16–4.71]; p &lt; 0.02)</td>
</tr>
<tr>
<td></td>
<td>Davé et al. [10]</td>
<td>90 diabetic patients with IS</td>
<td>Prospective observational (24 months)</td>
<td>CV ischemic events</td>
<td>CCL2 is an independent predictor of ischemic CV events, also in multivariate analysis (p &lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td>Yndestad et al. [15]</td>
<td>150 HF patients in NYHA class for &gt;4 months</td>
<td>Prospective observational (1 year)</td>
<td>Death, AMI and/or HF</td>
<td>CCL2 was associated with increased stroke incidence (p &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>Kim et al. [21]</td>
<td>616 patients with CAD</td>
<td>Prospective observational (24 months)</td>
<td>Death and/or CV events</td>
<td>CXCL16 was an independent predictor of adverse CV outcome (interquartile RR 1.271 [95% CI 1.025–1.577]; p = 0.02)</td>
</tr>
</tbody>
</table>

ACS = acute coronary syndrome; AMI = acute myocardial infarction; CAD = coronary heart disease; CI = confidence interval; CV = cardiovascular; HF = heart failure; HR = hazard ratio; IP-10 = interferon gamma-induced protein 10; IS = ischemic stroke; MACE = major acute coronary events; MIP-1 = monocyte inhibitor protein; MCP = monocyte chemoattractant protein; NYHA = New York heart association; OR = odds ratio; PTCA = percutaneous transluminal coronary angioplasty; RANTES = regulated on activation, normal T cell expressed and secreted; SA = stable angina; SDF = stromal cell-derived factor; UA = unstable angina.
CC chemokines

CCL2 was shown to increase plaque vulnerability, recruiting proinflammatory monocytes in both mouse [54] and human [55] atherosclerotic plaques. In an experimental model of myocardial ischaemia/reperfusion injury, CCL2 inhibition [56] or deletion [57] were shown to reduce infarct size. Similar findings were observed in mice deficient of CCR2 (CCL2 receptor) [58]. In addition to monocyte recruitment, CCL2 was shown to play a pivotal role in infarct healing, modulating macrophage differentiation and cytokine expression [59] and promoting fibroblast progenitor recruitment and differentiation [60]. In mouse models of stroke, CCL2 [61] or CCR2 [62] knockout mice resulted in a smaller infarct size. On the other hand, CCL2 was shown potentially to contribute to cerebral recovery, and differentiation [60]. In mouse models of stroke, 4. chemokine-binding proteins.

strategy approaches:

tigation were synthesized in accordance with different treatment with CCL5 triggered salvation intracellular strategies. Selective chemokine inhibitors currently under investigation were synthesized in accordance with different strategy approaches:

1. modified chemokines;

2. synthetic small molecules acting as antagonist or inverse agonist at chemokine receptors;

3. neutralising antibodies targeting chemokines or their receptors;

4. chemokine-binding proteins.

In addition, other drugs were shown to interfere indirectly with chemokine bioactivities.

Update on treatments targeting chemokines

Selective chemokines inhibitors

As reported in table 2 selective inhibitors of both CC and CXC chemokines have been recently tested in animal models of acute ischaemic cardiovascular diseases [65, 69-86].

Liew and coworkers investigated a nonagonist CCL2/MCP-1 mutant (PA508) with increased affinity for glycosaminoglycans, thus competing with CCL2 in binding CCR2. This molecule reduced myocardial ischaemia/reperfusion injury and limited neointima formation in experimental carotid artery injury [86, 74]. On the other hand, CCL5 inhibition was recently shown to be a very promising treatment against plaque vulnerability and acute myocardial infarction in mice. Brauneursreuther and coworkers showed that treatment with ['AANA'] RANTES (a mutated variant of CCL5/RANTES that inhibits chemokine oligomerisation on endothelial cell surface) reduced histological features of plaque vulnerability and infarct size in mice by impairing inflammatory cell recruitment [82, 87].

Related to the CXC chemokines, plerixafor (formerly AMD3100; Mozobil™) is a small bicyclam molecule originally developed for treatment of human immunodeficiency virus (HIV) infection and currently approved by US Food and Drug Administration and European Medicines Evaluation Agency (EMEA) for bone marrow-derived stem cell (BMSC) mobilisation in autologous stem cell transplantation. Plerixafor reversibly disrupts the interaction between chemokine receptor CXCR4 and its ligand CXCL12 [88]. The enhanced BMSC mobilisation improves haematological outcome but several insights suggest beneficial effects of plerixafor also in healing of ischaemia and ischaemia/reperfusion injury.

First in 2007, Proulx and coworkers reported that pulse therapy with AMD3100 in a rodent model of myocardial infarction reduced infarct size, improving systolic function [69]. Other research groups confirmed these findings in experimental models of both myocardial infarction [71, 75] and myocardial ischaemia/reperfusion injury [73], also reporting the key role played by BMSC recruitment in the recovery after ischaemic injury [71, 74]. AMD3100 has also proved to be effective in a mouse model of ischaemic stroke, where it reduced neutrophil recruitment, cytokine production and brain swelling, thus improving neurological outcome [76]. In contrast, when continuously infused, AMD3100 was shown to increase infarct size and impair cardiac remodelling, so worsening ventricular function [70-73]. These conflicting results might depend on the pharmacological properties of AMD3100 (especially reversible binding and the short plasma half-life [0.9 hours in rodents]). Thus, compared with pulse therapy, continuous infusion of AMD3100 would compromise BMSCs homing in to the injured myocardium, which is largely dependent on local expression of CXCL12.

Reparixin (an inhibitor of CXCR1 and CXCR2) at-
<table>
<thead>
<tr>
<th>Author et al.</th>
<th>Year</th>
<th>Animal</th>
<th>Model</th>
<th>Treatment and time to experiment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proux et al. [69]</td>
<td>2007</td>
<td>Rats</td>
<td>AMI</td>
<td>AM03100 1 mg/kg/day intraperitoneally or orally from 24 hours to 6 days after AMI (6 days)</td>
<td>Treatment reduced infarct size and improved systolic function (p &lt;0.05).</td>
</tr>
<tr>
<td>Morimoto et al. [70]</td>
<td>2007</td>
<td>Mice</td>
<td>AMI following by M-CSF administration</td>
<td>AM03100 continuous infusion 300 µg/kg/hr for 7 days after AMI (7 days)</td>
<td>Continuous infarction impaired infarction size and LV function.</td>
</tr>
<tr>
<td>Jujo et al. [71]</td>
<td>2010</td>
<td>Mice</td>
<td>AMI</td>
<td>AM03100 subcutaneous single dose 1 hour after AMI (28 days) After 28 days, acute treatment improved survival (p &lt;0.03) and cardiac remodelling (p &lt;0.05); treatment also increased EPCs mobilisation (p &lt;0.001). AM03100 increased the mobilisation of EPCs.</td>
<td></td>
</tr>
<tr>
<td>Dai et al. [72]</td>
<td>2010</td>
<td>Mice</td>
<td>AMI</td>
<td>AM03100 continuous infusion 47 µg/kg/hr from 24 hours before AMI until 21 days after (21 days)</td>
<td>Continuous infarction impaired cardiac remodelling (p &lt;0.05).</td>
</tr>
<tr>
<td>Huang et al. [73]</td>
<td>2011</td>
<td>Mice</td>
<td>IS (MCAO)</td>
<td>After 21 days, acute treatment improved survival (p &lt;0.03) and cardiac remodelling (p &lt;0.05); After 3 months, treated group showed significantly preserved LV free wall thickness, decreased infarct size and reduced LV dilatation (p &lt;0.05). Treatment reduced MPO-positive cell recruitment, proinflammatory cytokines (IL-6, TNF-α, IFN-γ) and brain γH</td>
<td></td>
</tr>
<tr>
<td>Garau et al. [77]</td>
<td>2005</td>
<td>Rats</td>
<td>Permanent and transient IS (MCAO)</td>
<td>After 3 hours, SSDF-1(S4V) improved cardiac function (p &lt;0.05). Treatment reduced MPO activity in both model of IS (p &lt;0.01) and infarct volume only in transient IS (p &lt;0.01).</td>
<td></td>
</tr>
<tr>
<td>Jang et al. [81]</td>
<td>2012</td>
<td>Mice</td>
<td>IS (MCAO)</td>
<td>After 30 min of ischaemia pre- or post-conditioning. (2 hours) Followed by 2 hours of reperfusion and ICAM-1 expression (p &lt;0.05). Treatment failed to impair neutrophil recruitment or improve survival or cardiac function.</td>
<td></td>
</tr>
<tr>
<td>Shen et al. [65]</td>
<td>2013</td>
<td>Rats</td>
<td>IS (MCAO)</td>
<td>Anti-CXCL1 antibody 50 µg/day intraperitoneally 10 min after ligation and 2 hours after reperfusion and daily for 7 days</td>
<td>Treatment reduced neutrophil recruitment and oxidative stress (p &lt;0.05). Anti-CXCL1 antibody decreased also MPO activity (p &lt;0.05) and ICAM-1 expression (p &lt;0.05).</td>
</tr>
<tr>
<td>Montecucco et al. [83]</td>
<td>2010</td>
<td>Mice</td>
<td>Carotid artery</td>
<td>Treatment reduced neutrophil recruitment and oxidative stress (p &lt;0.05) without improve infarct size, brain swelling or BBB permeability.</td>
<td></td>
</tr>
<tr>
<td>Oral et al. [85]</td>
<td>2013</td>
<td>Mice</td>
<td>AMI</td>
<td>Anti-CXCL1 antibody 50 µg/day 72 hours starting 4 days after AMI for 3 weeks</td>
<td>Treatment failed to impair neutrophil recruitment or improve survival or cardiac function.</td>
</tr>
<tr>
<td>Liñan et al. [86]</td>
<td>2010</td>
<td>ApoE−/− or ApoE−/−Ccr2−/− mice</td>
<td>Carotid injury</td>
<td>Anti-CXCL1 antibody 50 µg/day intraperitoneally 10 min after ligation and 2 hours after reperfusion and daily for 7 days</td>
<td>Treatment reduced neointima formation (p &lt;0.05) only in ApoE−/− mice.</td>
</tr>
</tbody>
</table>

AMI = acute myocardial infarction; BMI = body mass index; BBD = blood-brain barrier; BM = bone marrow; DPP = dipeptidyl peptidase; EPCs = endothelial progenitor cells; ICAM = intercellular adhesion molecule; IFN = interferon; IL = interleukin; IS = ischaemic stroke; LV = left ventricular; MCAO = middle cerebral arterial occlusion; M-CSF = monocyte colony stimulating factor; MMP = matrix metalloproteinases; MPO = myeloperoxidase; RANTES = regulated on activation, normal T cell expressed and secreted; ROS = reactive oxygen species; SDF-1 = stromal cell-derived factor 1; TNF = tumour necrosis factor.
Table 3
Nonselective treatments for acute ischemic events impacting the expression of chemokines and their receptors.

<table>
<thead>
<tr>
<th>Target</th>
<th>Author</th>
<th>Year</th>
<th>Animal</th>
<th>Model</th>
<th>Treatment (time to experiment)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBR2 agonist</td>
<td>Di Filippo et al. [90]</td>
<td>2004</td>
<td>Mice</td>
<td>2</td>
<td>5 min of ischaemia followed by 2 hours of reperfusion</td>
<td>WIN-55,212-2 3.5 mg/K/day intraperitoneally for 30 min before I/R induction (150 min)</td>
</tr>
<tr>
<td></td>
<td>Montecucco et al. [91]</td>
<td>2009</td>
<td>Mice</td>
<td>3</td>
<td>5 min of ischaemia followed by 24 hours of reperfusion</td>
<td>JWH-133 20 mg/kg 5 min before I/R induction</td>
</tr>
<tr>
<td></td>
<td>Mukhinat et al. [92]</td>
<td>2010</td>
<td>Mice</td>
<td>IS (MCAO)</td>
<td>1 mg/kg/day infusion for 3 days after onset of ischaemia (72 hours)</td>
<td>JWH-133 reduced infarct volume (p &lt;0.05). JWH-133 impaired CKCL2* induced neutrophil chemotaxis</td>
</tr>
<tr>
<td></td>
<td>Fernandez-Lopez et al. [93]</td>
<td>2012</td>
<td>Rats</td>
<td>IS (MCAO)</td>
<td>1 mg/kg twice daily (72 hours)</td>
<td>After 72 hours WIN55212-2 reduced infarct size (p &lt;0.01). After 24 hours treatment failed to reduce chemokine expression (CCKR, CCKR1, CCL2, CCL3)</td>
</tr>
<tr>
<td>DPP-4 inhibitors</td>
<td>Huber et al. [94]</td>
<td>2011</td>
<td>Mice</td>
<td>AMI</td>
<td>PTH 80 µg/kg/day for 6 days (30 days)</td>
<td>PTH improved cardiac function (p &lt;0.05) whereas coadministration of AM0100 neutralised this improvement. As DPP-4 inhibitor; PTH increased CKCL2, enhancing recruitment of BM-derived EPCs</td>
</tr>
<tr>
<td>Oestrogen</td>
<td>Zhang et al. [95]</td>
<td>2010</td>
<td>Ovariectomised mice</td>
<td>IS (MCAO)</td>
<td>G1 1.8 mg/day (96 hours)</td>
<td>Treatment reduced infarct size (p &lt;0.05); in addition CKCL2 was reduced (p &lt;0.05) whereas CCR7 was up-regulated (p &lt;0.05)</td>
</tr>
<tr>
<td></td>
<td>Chen et al. [96]</td>
<td>2012</td>
<td>Ovariectomised rats</td>
<td>AMI</td>
<td>17β-oestradiol 0.1 or 1 mg/kg (24-hours, 1, 3 and 28 days)</td>
<td>Oestradiol increased BMSCs, CCL12 and capillary density in myocardium (p &lt;0.05)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Chen et al. [97]</td>
<td>2012</td>
<td>Castrated rat</td>
<td>AMI</td>
<td>Testosterone 2 mg/kg/day (1, 3 and 28 days)</td>
<td>Testosterone increased BMSCs, CCL12 and capillary density in myocardium (p &lt;0.05)</td>
</tr>
<tr>
<td></td>
<td>Iwata et al. [99]</td>
<td>2006</td>
<td>Humans</td>
<td>Patients with AMI undergoing PTCA and stenting</td>
<td>Pravastatin or atorvastatin (different doses) (6 months)</td>
<td>During follow-up chemokine decreased without significant differences between the two groups (CCR2, CCKR2, and CCKR3). Treatment did not prevent coronary restenosis</td>
</tr>
<tr>
<td></td>
<td>Sironi et al. [98]</td>
<td>2006</td>
<td>Rats</td>
<td>IS (MCAO)</td>
<td>Simvastatin 20 mg/Kg subcutaneously 3 days before IS onset (2 and 24 hours)</td>
<td>Statin reduced infarct size and CCL2 (p &lt;0.05)</td>
</tr>
<tr>
<td>Statin</td>
<td>De Lemos et al. [8]</td>
<td>2007</td>
<td>Humans</td>
<td>ACS</td>
<td>Simvastatin 0.5 mg/kg and or BMSCs 1 or 3 x 10⁶ intravenous 24 hours after stroke (7 days)</td>
<td>After 4 months statin treated group had smaller increase in CCL2 levels (p = 0.005) and better outcome</td>
</tr>
<tr>
<td></td>
<td>Cui et al. [100]</td>
<td>2009</td>
<td>Rats</td>
<td>IS (MCAO)</td>
<td>Simvastatin 0.5 mg/kg and BMSCs 1 or 3 x 10⁶ (4 months)</td>
<td>Combined treatment improved neurological outcome (p &lt;0.05) and increased angiogenesis (p &lt;0.05) up-regulating CKCL2 and CCKR4 on BMSCs surface (p &lt;0.05).</td>
</tr>
<tr>
<td></td>
<td>Qi et al. [101]</td>
<td>2012</td>
<td>Rats</td>
<td>AMI</td>
<td>Atorvastatin 10 mg/kg/day alone or associated with AMD3100 (7 days)</td>
<td>Statin treated group has increase in NO, CKCL2 and CCKR4 expression (p &lt;0.05). And AMD3100 offset these effects</td>
</tr>
<tr>
<td>PPAR-γ agonist</td>
<td>Wayman et al. [102]</td>
<td>2002</td>
<td>Rats</td>
<td>25 min of ischaemia followed by 2 hours of reperfusion</td>
<td>Several PPAR-γ agonist (2 hours)</td>
<td>Pioglitazone reduced infarct size (p &lt;0.05). 15d-PGJ2 was associated with decreased infarct size and reduction in CCL2 mRNA expression (p &lt;0.05).</td>
</tr>
<tr>
<td></td>
<td>Ito et al. [103]</td>
<td>2003</td>
<td>Rats</td>
<td>30 min of ischaemia followed by 24 hours of reperfusion</td>
<td>Pioglitazone 3 mg/kg/day 7 days before onset of AMI (24 hours)</td>
<td>Treated group showed smaller infarct size (p &lt;0.05) and lower CCL2 mRNA (p &lt;0.05).</td>
</tr>
<tr>
<td>ROS scavenger</td>
<td>Nakamura et al. [104]</td>
<td>2009</td>
<td>Humans</td>
<td>AMI</td>
<td>Edaravone 30 mg intravenously before reperfusion (24 hours, 3, 5, 7, 14 days)</td>
<td>Edaravone was associated to decreased circulating CCL2 (p &lt;0.05), improved LVEF and reduced rehospitalisation (p &lt;0.05). ROS scavenger</td>
</tr>
<tr>
<td>Nampt inhibitor</td>
<td>Montecucco et al. [105]</td>
<td>2013</td>
<td>Mice</td>
<td>30 min of ischaemia followed by 24 hours of reperfusion</td>
<td>FKB66 30 mg/kg intraperitoneally 5 min after ischaemia and 12 h after reperfusion onset (1, 3 and 24 hours)</td>
<td>FKB66 reduced infarct size after 24 hours associated to reduced neutrophil infiltration ROS production and CKCL2* (p &lt;0.01)</td>
</tr>
</tbody>
</table>

*In murine model CXCL2 is referred to human CXCL8 (homologue of murine CXCL2)

ACS = acute coronary syndrome; AMI = acute myocardial infarction; BMSC = bone marrow stromal cell; CBR = cannabinoid receptor; DPP = dipeptidyl peptidase; IS = ischaemic stroke; I/R = ischaemia/reperfusion injury; IS = ischaemic stroke; LVEF = left ventricular ejection fraction; MCAO = middle cerebral artery occlusion; Nampt = nicotinamide phosphoribosyltransferase; NO = nitric oxide; PPAR = peroxisome proliferator-activated receptors; PTCA = percutaneous transluminal coronary angioplasty; PTH = parathyroid hormone; ROS = reactive oxygen species.
tenuates neutrophil recruitment (assessed as myeloperoxidase activity) in a rodent model of stroke. However, the promising preliminary results have been weakened by more recent conflicting results on the pathophysiological relevance of neutrophils in cerebral infarction [77–79].

Evasins (chemokine-binding proteins secreted in the saliva of bloodsucking parasites, such as ticks) have been recently isolated and tested in acute cardiovascular diseases [89]. We showed that treatment with evasin-3 (an inhibitor of CXC chemokines) was able to reduce the recruitment of leucocytes in the injured tissues in mouse models of myocardial infarction [83] and ischaemic stroke [84]. However, the potent anti-inflammatory properties of evasin-3 were associated with improvements in infarct size only in acute myocardial ischaemia. Conversely, the selective inhibition of CXCL1 failed to reduce neutrophil recruitment or infarct size in a mouse model of chronic myocardial ischaemia [85].

Nonselective chemokine inhibitors

Different drugs were shown to modulate indirectly chemokines and their cognate receptors in ischaemic tissues, thus interfering with post-infarction inflammation and ischaemia/reperfusion injury [8, 90–105] (table 3).

For instance, Di Filippo and coworkers showed an improvement in a model of ischaemia/reperfusion myocardial injury under treatment with the cannabinoid receptor type 2 (CBR2) agonist WIN-55,212-2, associated with a decreased CXCL2 expression [90]. Accordingly, we and other researchers [91, 92] showed that treatment with the CBR2 agonist JWH-133 was able to reduce myocardial infarct size and the associated increase of the chemokines CXCL1, CXCL2 and CCL3. Dipeptidyl peptidase-4 ([DPP-4], a serine protease that cleaves off N-terminal dipeptides from peptide substrate) was shown to improve cardiac function after myocardial ischaemia, increasing CXCL12-mediated BMSC recruitment [80, 94].

Finally, the inhibition of chemokine up-regulation after acute myocardial infarction was also induced by the reactive oxygen species (ROS) scavenger edaravone [104] and FK866 (a nicotinamide phosphoribosyltransferase [Nampt] inhibitor) [105].

Limitations of antichemokine treatments in humans

To date, only two chemokine receptor antagonists have been approved by the US Food and Drug Administration and the EMEA: the CCR5 antagonist maraviroc for treatment of HIV and the CXCR4 antagonist plerixafor for stem cell mobilisation. There were many disappointments in clinical testing of potential inhibitors of chemokines and their receptors. The compounds might have failed for several reasons, especially during clinical evaluation, that point out the differences between animal models and humans. Although the redundancy in the chemokine system can explain lack of efficiency or adverse drug reactions [106], the greatest concerns arise from immunological side effects that impair host defenses. The pivotal role of chemokines in immune the response against pathogens has been well established [107]. Similarly, impairment in immune responses was observed after inhibition of the CCL2–CCR2 axis [107] or after Met-CCL5 administration [108]. In addition, animals used for experiments are usually maintained in a pathogen-free environment, an uncommon situation in human life. It is conceivable that side effects are comparable to those caused by prolonged treatment with tumor necrosis factor blockers [109] or corticosteroids [110].

Moreover, both modified chemokines and synthetic peptides have poor bioavailability orally and require subcutaneous or intravenous administration. This might increase the risk of developing allergic reactions or antibodies that would hamper long-term treatment.

Conclusion

It is well established that the chemokine system plays a pivotal pathophysiological role in cardiac and cerebral ischaemic injuries, modulating a wide range of biological processes (especially leucocyte recruitment, but also angiogenesis and BMSC infiltration). However, the biological consequences of their pharmacological inhibition require further basic research before the clinical use. As biomarkers, chemokines might also play a critical role in the better assessment of cardiovascular risk. In that case, additional clinical studies are also needed to validate their potential to predict acute ischaemic cardiovascular events in both primary and secondary prevention.

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