Antichemokine treatments in acute ischaemic cardiovascular diseases

Federico Carbone[a,b], *Fabrizio Montecucco*[a,b]

[a] Division of Cardiology Department of Internal Medicine, University of Geneva, Switzerland

[b] First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa School of Medicine. IRCCS Azienda Ospedaliera Universitaria San Martino–IST Istituto Nazionale per la Ricerca sul Cancro, Italy

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-

Funding / potential competing interests: This work was supported by Swiss National Science Foundation Grant to Dr. F. Montecucco (#32003B_134963/1). The authors have no conflict of interest.

Abbreviations

BMSCs:	bone marrow-derived stem cells
CBR:	cannabinoid receptor
CT:	computed tomography
CV:	cardiovascular
DPP:	dipeptidyl peptidase
ELR:	glutamate-leucine-arginine
EMEA:	European Medicines Evaluation Agency
EPCs:	endothelial progenitor cells
GLP:	glucagon-like peptide
HIV:	human immunodeficiency virus
MCP:	monocyte chemoattractant protein
MIP:	macrophage inflammatory protein
MMP:	matrix metalloproteinase
mRNA	messsenger ribonucleic acid
oxLDL:	oxidised low density lipoprotein
PPAR:	peroxisome proliferator-activated receptor
RANTES:	regulated upon activation normal T-cell expressed and secreted
ROS:	reactive oxygen species
SDF:	stromal cell-derived factor

Correspondence:
Fabrizio Montecucco, MD, PhD
Cardiology Division, Foundation for Medical Researches
Department of Internal Medicine, University of Geneva
64 Avenue Roseraie
CH-1211 Geneva
Switzerland
fabrizio.montecucco[at]unige.ch

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. CX3CL1/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 potent 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 contast to other CXC chemokines, CXCL12 and its receptors CXCX4 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 1
Summary of studies investigating chemokine circulating levels as predictors of acute ischemic events in humans.

Chemokine	Author	Population	Study design (follow-up)	Outcome	Findings
CCL2/MCP-1	De Lemos et al. [5]	2270 from OPUS-TIMI 16 trial	Prospective observational (10 months)	Death and/or AMI	CCL2 was independent predictor of worst outcome (HR 1.42 [95% CI 1.021.98]; p = 0.04)
	Kervinen et al. [6]	183 patients with chest pain (64 SA, 60 UA and 59 AMI)	Prospective observational (13 months)	CV events	Increased CCL2 was associated with increased risk (OR 1.85 [95% CI 1.16–4.71]; p <0.02)
	Haim et al. [7]	233 CAD patients with recurrent CV events compared to a control group	Two nested case-control study (–)	CV events	CCL2 was not associated with long-term CV risk
	De Lemos et al. [8]	3467 patients with ACS from A to Z trial	Prospective randomised trial (4 months)	Death, AMI and/or HF	Higher level of CCL2 was an independent predictor for death (interquartile HR 1.34 [95% CI 1.01–1.77]; p <0.0001)
	Kuriyama et al. [9]	197 subjects (89 IS; 18 recurrent IS and 90 controls)	Case-control study	Recurrent IS	CCL2 was associated with increased stroke incidence (p <0.05)
	Daví et al. [10]	90 diabetic patients with IS	Prospective observational (24 months)	CV ischemic events	CCL2 is an independent predictor of ischaemic CV events, also in multivariate analysis (p <0.001)
	Frazier et al. [11]	490 stenting undergoing PTCA + stenting	Prospective observational (1 year)	MACE	Higher level of CCL2 was an independent predictor for MACE (p <0.05)
CCL3/MIP-1α	De Jager et al. [12]	55UA patients	Prospective observational (180 days)	CV events	Higher CCL3 quartile was predictive for occurrence of ACS (p <0.01)
CCL4/MIP-1β	Tatara et al. [13]	551 hypertensive patients	Prospective observational (37.2 months)	IS and CV disease	CCL4 was an independent risk factor for worse outcome (interquartile HR 3.59 [95% CI 1.01–12.69]; p = 0.047)
CCL5/RANTES	Cavusoglu et al. [14]	389 male undergoing PTCA	Prospective observational (2 years)	Cardiac death and AMI	CCL5 was an independent predictor of cardiac death and AMI occurrence in all subgroups (p <0.05).
CCL19 CCL21	Yndestad et al. OPTIMAAL trial [15]	150 HF patients in NYHA class for >4 months	Prospective observational (1 year)	Cardiac death	CCL21 levels were an independent predictor of mortality in multivariate analysis (HR 2.72 [95% CI 1.01–7.36]; p = 0.048)
CCL23/eotaxin-3	Falcone et al. [16]	1014 patients with CAD	Prospective observational (4.1 years)	CV events	Lower CCL26 was an independent predictor of adverse CV events (HR 0.42 [95% CI 0.29–0.61]; p <0.001)
CCL5/RANTES CXCL10/IP-10 CCL11/eotaxin-1 CCL2/MCP-1	Canouï-Poitrine et al. PRIME study [17]	9771 men (621 CAD and 1242 controls)	Two nested case-control study (10 years)	CAD (SA or ACS)	None of four chemokines were independent predictors of CAD
		(95 IS and 190 controls)		IS	CCL5 (HR 1.70 [95% CI 1.05–2.74]; p <0.05), CXCL10 (HR 1.53 [95% CI 1.06–2.20], p <0.05) and CCL11 (HR 1.59 [95% CI 1.02–2.46]; p <0.05) but not CCL-2 are predictors of IS
CXCL12/SDF-1	Schutt et al. [18]	206 subject referred for elective coronary angiography	Prospective observational (1 year)	IS	CXCL12 was higher in patients who suffered an IS (p = 0.007) and an independent predictor for IS (HR 15.29 [95% CI 3.05–76.71]: p <0.05)
	Kim et al. [19]	104 IS patients	Prospective observational (3 months)	Long-term outcome	CXCL12 was associated with infarct size (p = 0.02) and independently predicted better outcome (OR 1.16 [95% CI 1.02–1.31]; p = 0.008)
	Fortunato et al. [20]	175 patients after AMI	Prospective observational (1 year)	Cardiac death, recurrent AMI and/or HF	CXCL12 was an independent predictor of worst outcome (OR 3.8 [95% CI 1.44–10.19]; p = 0.007)
CXCL16	Tan et al. [21]	616 patients with CAD	Prospective observational (24 months)	Death and/or CV events	CXCL16 was an independent predictor of adverse outcome (interquartile RR 1.271 [95% CI 1.025–1.577]; p = 0.02)
	Ueland et al. [22]	244 patients with IS	Prospective observational (47 months)	CV mortality	CXCL16 was an independent predictor of CV mortality in multivariate analysis (HR 1.69 [95% CI 1.20–2.40]; p = 0.003)

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 = ischaemic 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, promoting recruitment of bone marrow-derived stromal cells [63] and newly formed neuroblasts from the neurogenic region [64]. Interestingly, these latter findings suggest a double role of CCL2 in ischaemic stroke, harmful in the earlier stages and protective later.

Another CC chemokine (CCL5) was recently shown to have detrimental effects in ischaemia/reperfusion injuries [65]. CCL5 orchestrates the recruitment of several inflammatory cell subsets, such as monocytes, neutrophils, dendritic cells, and lymphocytes, to the inflammatory site, through the binding to various transmembrane receptors (CCR1, 3, 4, 5). As showed by our research group, treatment with antibodies neutralising CCL5 bioactivity reduced both infarct size and postinfarction heart failure in a mouse model of chronic cardiac ischaemia [66]. On the other hand, in experimental models of focal cerebral ischaemia, deletion of CCL5 was associated with reduction in leucocyte infiltration, infarct size and blood-brain barrier permeability [67]. In addition, Tokami and coworkers, starting from the observation that CCL5 serum levels were increased in ischaemic stroke patients as compared with healthy controls ($p < 0.001$), reported that CCL5 expression was up-regulated in mouse neurones after middle cerebral artery occlusion [68]. In the same study, the authors observed that treatment with CCL5 triggered salvation intracellular pathways in experimental neuronal cells [68].

Update on treatments targeting chemokines

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.

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].

Liehn 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. Braunersreuther and coworkers showed that treatment with [^{44}AANA^{47}]-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 2
Summary of selective anti-chemokine treatments for acute ischemic events in animal models.

Author	Year	Animal	Model	Treatment and time to experiment	Outcome
Proulx et al. [69]	2007	Rats	AMI	AMD3100 1 mg/kg/day intraperitoneally or orally from 24 hours to 6 days after AMI (6 days)	Treatment reduced infarct size and improved systolic function ($p < 0.05$).
Morimoto et al. [70]	2007	Mice	AMI following by M-CSF administration	AMD 3100 continuous infusion 300 µg/kg/h for 7 days after AMI (7 days)	Continuous infusion impaired infarction size and LV function.
Jujo et al. [71]	2010	Mice	AMI	AMD 3100 subcutaneous single dose 1 hour after AMI (28 days)	After 28 days acute treatment improved survival ($p < 0.03$) and cardiac remodelling ($p < 0.05$); treatment also increased EPCs mobilisation ($p < 0.001$).
				AMD 3100 2.5 mg/week continuous infusion for 2 weeks after AMI (14 days)	After 14 days continuous infusion impaired cardiac remodelling ($p < 0.05$).
Dai et al. [72]	2010	Mice	AMI	AMD3100 continuous infusion 47 µg/kg/h from 24 hours before AMI until 21 days after (21 days)	Continuous infusion impaired cardiac remodelling ($p < 0.05$).
Huang et al. [73]	2011	Mice	25 min of global ischaemia followed by 40 min of reperfusion	AMD3100 continuous infusion 5 µg/ml from 5 min before ischaemia until 80 min after (80 min)	Continuous infusion impaired cardiac remodelling ($p < 0.05$).
Jujo et al. [74]	2013	Mice	Reperfusion after 60 min of ischaemia	AMD3100 subcutaneous injection 5 mg/kg (3, 7, 14 and 28 days)	After 3 days treatment reduced infarct size ($p < 0.05$); after 4 weeks improved cardiac function ($p < 0.05$). AMD3100 increased the mobilisation of EPCs.
Luo et al. [75]	2013	Rats	AMI	AMD3100 1 mg/kg/day intraperitoneally 1 or 24 hours post-AMI (6 and 24 hours, 7 days and 3 months)	After 3 months treated group showed significantly preserved LV free wall thickness, decreased infarct size and reduced LV dilatation ($p < 0.05$).
Huang et al. [76]	2013	Mice	IS (MCAO)	AMD3100 1 mg/kg/day intraperitoneally for 3 days after IS (24, 48 and 72 hours after IS)	Treatment reduced MPO-positive cell recruitment, proinflammatory cytokines (IL-6, TNF-α, IFN-γ) and brain oedema ($p < 0.005$). After 3 days treated group has improved neurological score ($p < 0.05$).
Garau et al. [77]	2005	Rats	Permanent and transient IS (MCAO)	Reparixin 15 mg/kg intravenously at the time of ischaemia and every 2 hours for four times (24 hours)	Treatment reduced MPO activity in both model of IS ($p < 0.01$) and infarct volume only in transient IS ($p < 0.01$).
Villa et al. [78]	2007	Rats	IS (MCAO)	Reparixin 15 mg/kg intravenously 2 and 4 hours after reperfusion (2, 24, 48 hours)	Reparixin reduced infarct size ($p < 0.05$) and improve neurological deficit ($p < 0.05$).
Sousa et al. [79]	2013	Mice	IS (MCAO)	Reparixin 30 mg/kg subcutaneously 60 minutes before ischaemia onset (24 hours)	Treated mice reduce inflammatory infiltration assessed as MPO activity ($p < .0.05$)
Kanki et al. [80]	2011	Rats	30, 45, 75 or 90 min of ischaemia followed by 2 hours of reperfusion	MMP-2/DPP-4–resistant form of SDF-1 [SSDF-1(S4V)] (10, 30 min, 4, 24 hours)	After 3 hours SSDF-1(S4V) improved cardiac function ($p < 0.05$).
Jang et al. [81]	2012	Rats	30 min of ischaemia followed by 2 hours of reperfusion	Recombinant CXCL12 or AMD3100 or nothing: continuous infusion, or ischaemic pre- or post-conditioning. (2 hours)	Continuous infusion or preischaemic exposure of recombinant CXCL12 resulted in a dramatic reduction of infarct size ($p < 0.05$).
Braunersreuther et al. [82]	2010	$ApoE^{-/-}$ and/or $Ccr5^{-/-}$ mice	30 min of ischaemia followed by reperfusion	[44AANA47]-RANTES 10 µg/kg intraperitoneally 5 minutes before reperfusion (24 hours)	Treatment reduced leucocyte infiltration, oxidative stress and apoptosis ($p < 0.05$). Treated group had reduced infarct size in $ApoE^{-/-}$ ($p < 0.05$) but not in $ApoE^{-/-}$ $Ccr5^{-/-}$ mice.
Shen et al. [65]	2013	Rats	30 min of ischaemia followed by 2 hours of reperfusion	Anti-CCR5 antibody 0.2 mg/kg or CCR5 agonist 0.1 mg/kg intravenously 20 min after ischaemia (2 hours)	CCR agonist treatment increased infarct size compared to control ($p < 0.05$) whereas anti-CCR5 decreased necrotic area ($p < 0.05$). Anti-CCR5 antibody decreased also MPO activity ($p < 0.05$) and ICAM-1 expression ($p < 0.05$).
Montecucco et al. [83]	2010	Mice	30 min of ischaemia followed by reperfusion	Evasin 3 5 mg/ml 5 minutes after onset of ischaemia (24 hours)	After 8 hours evasin-3 reduced infarct size ($p < 0.05$) in association with decreased neutrophil recruitment and ROS production ($p < 0.05$). Not been proved activation of cardioprotective pathways.
Copin et al. [84]	2013	$ApoE^{-/-}$ mice	Constrictive stenosis of carotid artery	Evasin-3 1 µg/day 6 weeks after cast implantation (21 days)	Treatment reduced neutrophil recruitment and MMP-9 content in atherosclerotic plaque ($p < 0.05$) associated to a weak increase in collagen.
		Wild-type mice	MCAO	Evasin-3 10 µg 5 minutes after onset of ischaemia (24 hours)	Treatment reduced neutrophil recruitment and oxidative stress ($p < 0.05$) without improve infarct size, brain swelling or BBB permeability.
Oral et al. [85]	2013	Mice	AMI	Anti-CXCL1 antibody 50 µg/72 hours starting 4 days after AMI for 3 weeks	Treatment failed to impair neutrophil recruitment or improve survival or cardiac function.
Liehn et al. [86]	2010	Mice	30 min of ischaemia followed by reperfusion	PA508 0, 1, 5, or 10 µg/day intraperitoneally 10 min after ligation and 2 hours after reperfusion and daily for 7 days	10 µg/day preserved global LV function, both contractility and relaxation ($p < 0.05$).
		$ApoE^{-/-}$ or $ApoE^{-/-}$ $Ccr2^{-/-}$ mice	Carotid wire injury	PA508 0, 1, 5, or 10 µg/day intraperitoneally 1 day before injury and daily for 3 weeks	Treatment reduced neointima formation ($p < 0.05$) only in $ApoE^{-/-}$ mice.

AMI = acute myocardial infarction; BBB = blood-brain barrier; BM bone marrow; DDP = 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.

Target	Author	Year	Animal	Model	Treatment (time to experiment)	Outcome
CBR2 agonist	Di Filippo et al. [90]	2004	Mice	25 min of ischaemia followed by 2 hours of reperfusion	WIN-55,212-2 3.5 mg/K/day intraperitoneally 30 min before I/R induction (150 min)	WIN55212-2 improved myocardial injury and this effect was reduced by pre-treatment with a CBR2 but not CBR1 antagonist. WIN55212-2 reduced CXCL2*
	Montecucco et al. [91]	2009	Mice	30 min of ischaemia followed by 24 hours of reperfusion	JWH-133 20 mg/kg 5 min before I/R induction	JWH-133 reduced infarct size compared with control ($p < 0.05$) and treatment with CBR2 antagonist ($p < 0.01$). JWH-133 reduced CXCL1, CXCL2* and CCL3 ($p < 0.05$).
	Murikinati et al. [92]	2010	Mice	IS (MCAO)	JWH-133 1 mg/kg/day continuous infusion for 3 days after onset of ischaemia (72 hours)	JWH-133 reduced infarct volume ($p < 0.05$). JWH-133 impaired CXCL2*-induced neutrophil chemotaxis
	Fernandez-Lopez et al. [93]	2012	Rats	IS (MCAO)	WIN-55,212-2 1 mg/kg twice daily (72 hours)	After 72 hours WIN55212-2 reduced infarct size ($p < 0.01$). After 24 hours treatment failed to reduce chemokine expression (CCRR, CX3CR1, CCL2, CCL3)
DPP-4 inhibitors	Huber et al. [94]	2011	Mice	AMI	PTH 80 µg/kg/day per 6 days (30 days)	PTH improved cardiac function ($p < 0.05$) whereas coadministration of AMD3100 neutralised this improvement. As DPP-4 inhibitor, PTH increased CXCL12, enhancing recruitment of BM-derived EPCs
Oestrogen	Zhang et al. [95]	2010	Ovariectomised mice	IS (MCAO)	Oestrogen receptor agonist, G1 1.8 mg/day (96 hours)	Treatment reduced infarct size ($p < 0.05$); in addition CXCL2 was reduced ($p < 0.05$) whereas CCR7 was up-regulated ($p < 0.05$)
	Chen et al. [96]	2012	Ovariectomised rats	AMI	17-β-oestradiol 0.1 or 1 mg/kg (24 hours, 1, 3 and 28 days)	Oestradiol increased BMSCs, CCL12 and capillary density in myocardium ($p < 0.05$)
Testosterone	Chen et al. [97]	2012	Castrated rat	AMI	Testosterone 2 mg/kg/day (1, 3 and 28 days)	Testosterone increased BMSCs, CCL12 and capillary density in myocardium ($p < 0.05$)
Statin	Iwata et al. [99]	2006	Humans	Patients with AMI undergoing to PTCA + stenting	Pravastatin or atorvastatin (different doses) (6 months)	During follow-up chemokine decreased without significant differences between the two groups (CCR2, CXCR2, and CXCR3). Treatment did not prevent coronary restenosis
	Sironi et al. [98]	2006	Rats	IS (MCAO)	Simvastatin 20 mg/Kg subcutaneously 3 days before IS onset (2 and 24 hours)	Statin reduced infarct size and CCL2 ($p < 0.05$)
	De Lemos et al. [8]	2007	Humans	ACS	Simvastatin 40 mg/day for 1 months followed by 80 mg/day (4 months)	After 4 months statin treated group had smaller increase in CCL2 levels ($p = 0.005$) and better outcome
	Cui et al. [100]	2009	Rats	IS (MCAO)	Simvastatin 0.5 mg/kg and or BMSCs 1 or 3x106 intravenous 24 hours after stroke (7 days)	Combined treatment improved neurological outcome ($p < 0.05$) and increased angiogenesis ($p < 0.05$) up-regulating CXCL12 andCXCR4 on BMSCs surface ($p < 0.05$).
	Qiu et al. [101]	2012	Rats	AMI	Atorvastatin 10 mg/kg/day alone or associated with AMD3100 (7 days)	Statin treated group has increase in NO, CXCL12 and CXCR4 expression ($p < 0.05$). AMD3100 offset these effects
PPAR-γ agonist	Wayman et al. [102]	2002	Rats	25 min of ischaemia followed by 2 hours of reperfusion	Several PPAR-γ agonist (2 hours)	Pioglitazone reduced infarct size ($p < 0.05$). 15d PGJ2 was associated with decreased infarct size and reduction in CCL2 mRNA expression ($p < 0.05$).
	Ito et al. [103]	2003	Rats	30 min of ischaemia followed by 24 hours of reperfusion	Pioglitazone 3 mg/kg/day 7 days before onset of AMI (24 hours)	Treated group showed smaller infarct size ($p < 0.05$) and lower CCL2 mRNA ($p < 0.05$).
ROS scavenger	Nakamura et al. [104]	2009	Humans	AMI	Edaravone 30 mg intravenously before reperfusion (24 hours, 3, 5, 7, 14 days)	Edaravone was associated to decreased circulating CCL2 ($p < 0.05$), improved LVEF and reduced rehospitalisation ($p < 0.05$).
Nampt inhibitor	Montecucco et al. [105]	2013	Mice	30 min of ischaemia followed by 24 hours of reperfusion	FK866 30 mg/kg intraperitoneally 5 min after ischaemia and 12 h after reperfusion onset (1, 3 and 24 hours)	FK866 reduced infarct size after 24 hours associated to reduced neutrophil infiltration ROS production and CXCL2* ($p < 0.01$)

*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; 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 (CBR_2) agonist WIN-55,212-2, associated with a decreased CXCL2 expression [90]. Accordingly, we and other researchers [91, 92] showed that treatment with the CBR_2 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 substrates) 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.

References

1 Magnussen CG, Niinikoski H, Juonala M, Kivimaki M, Ronnemaa T, Viikari JS, et al. When and how to start prevention of atherosclerosis? Lessons from the Cardiovascular Risk in the Young Finns Study and the Special Turku Coronary Risk Factor Intervention Project. Pediatr Nephrol 2012;27:1441–52.
2 Borden WB, Davidson MH. Updating the assessment of cardiac risk: beyond Framingham. Rev Cardiovasc Med 2009;10:63–71.
3 Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. Circulation 2003;108: 1664–72.
4 Imanishi T, Akasaka T. Biomarkers associated with vulnerable atheromatous plaque. Curr Med Chem 2012;19:2588–96.
5 de Lemos JA, Morrow DA, Sabatine MS, Murphy SA, Gibson CM, Antman EM, et al. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation 2003;107:690–5.
6 Kervinen H, Manttari M, Kaartinen M, Makynen H, Palosuo T, Pulkki K, et al. Prognostic usefulness of plasma monocyte/macrophage and T-lymphocyte activation markers in patients with acute coronary syndromes. Am J Cardiol 2004;94:993–6.

7 Haim M, Tanne D, Boyko V, Reshef T, Goldbourt U, Battler A, et al. Monocyte chemoattractant protein-1 and recurrent cardiovascular events in patients with stable coronary heart disease. Clin Cardiol 2005;28: 31–5.
8 de Lemos JA, Morrow DA, Blazing MA, Jarolim P, Wiviott SD, Sabatine MS, et al. Serial measurement of monocyte chemoattractant protein-1 after acute coronary syndromes: results from the A to Z trial. J Am Coll Cardiol 2007;50:2117–24.
9 Kuriyama N, Mizuno T, Kita M, Nagakane Y, Hosomi A, Harada S, et al. Predictive markers of blood cytokine and chemokine in recurrent brain infarction. J Interferon Cytokine Res 2009;29:729–34.
10 Davi G, Tuttolomondo A, Santilli F, Basili S, Ferrante E, Di Raimondo D, et al. CD40 ligand and MCP-1 as predictors of cardiovascular events in diabetic patients with stroke. J Atheroscler Thromb 2009;16:707–13.
11 Frazier L, Vaughn WK, Willerson JT, Ballantyne CM, Boerwinkle E. Inflammatory protein levels and depression screening after coronary stenting predict major adverse coronary events. Biol Res Nurs 2009; 11:163–73.
12 de Jager SC, Kraaijeveld AO, Grauss RW, de Jager W, Liem SS, van der Hoeven BL, et al. CCL3 (MIP-1 alpha) levels are elevated during acute coronary syndromes and show strong prognostic power for future ischemic events. J Mol Cell Cardiol 2008;45:446–52.
13 Tatara Y, Ohishi M, Yamamoto K, Shiota A, Hayashi N, Iwamoto Y, et al. Macrophage inflammatory protein-1beta induced cell adhesion with increased intracellular reactive oxygen species. J Mol Cell Cardiol 2009; 47:104–11.
14 Cavusoglu E, Eng C, Chopra V, Clark LT, Pinsky DJ, Marmur JD. Low plasma RANTES levels are an independent predictor of cardiac mortality in patients referred for coronary angiography. Arterioscler Thromb Vasc Biol 2007;27:929–35.
15 Yndestad A, Finsen AV, Ueland T, Husberg C, Dahl CP, Oie E, et al. The homeostatic chemokine CCL21 predicts mortality and may play a pathogenic role in heart failure. PLoS One 2012;7:e33038.
16 Falcone C, Minoretti P, D'Angelo A, Buzzi MP, Coen E, Emanuele E, et al. Markers of eosinophilic inflammation and risk prediction in patients with coronary artery disease. Eur J Clin Invest 2006;36:211–7.
17 Canoui-Poitrine F, Luc G, Mallat Z, Machez E, Bingham A, Ferrieres J, et al. Systemic chemokine levels, coronary heart disease, and ischemic stroke events: the PRIME study. Neurology 2011;77:1165–73.
18 Schutt RC, Burdick MD, Strieter RM, Mehrad B, Keeley EC. Plasma CXCL12 levels as a predictor of future stroke. Stroke 2012;43:3382–6.
19 Kim YS, Baek W, Kim MK, Kim HY, Lee KY, Lee YJ, et al. Association between serum stromal cell-derived factor-1alpha and long-term outcome of acute ischemic stroke. Eur Neurol 2012;67:363–9.
20 Fortunato O, Spinetti G, Specchia C, Cangiano E, Valgimigli M, Madeddu P. Migratory activity of circulating progenitor cells and serum SDF-1alpha predict adverse events in patients with myocardial infarction. Cardiovasc Res 2013. doi: 10.1093/cvr/cvt153.
21 Tan K, Lu S, Chen Y, Song X, Wu X, Jin Z, et al. CXC chemokine ligand 16 as a prognostic marker in patients with intermediate coronary artery lesions: a 2-year follow-up study. Tohoku J Exp Med 2011;223: 277–83.
22 Ueland T, Smedbakken LM, Hallen J, Atar D, Januzzi JL, Halvorsen B, et al. Soluble CXCL16 and long-term outcome in acute ischemic stroke. Atherosclerosis 2012;220:244–9.
23 Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med 2006;354:610–21.
24 Rotondi M, Chiovato L. The chemokine system as a therapeutic target in autoimmune thyroid diseases: a focus on the interferon-gamma inducible chemokines and their receptor. Curr Pharm Des 2011;17: 3202–16.
25 Frangogiannis NG. Chemokines in ischemia and reperfusion. Thromb Haemost 2007;97:738–47.
26 Saxena A, Fish JE, White MD, Yu S, Smyth JW, Shaw RM, et al. Stromal cell-derived factor-1alpha is cardioprotective after myocardial infarction. Circulation 2008;117:2224–31.
27 Morimoto H, Hirose M, Takahashi M, Kawaguchi M, Ise H, Kolattukudy PE, et al. MCP-1 induces cardioprotection against ischaemia/reperfusion injury: role of reactive oxygen species. Cardiovasc Res 2008;78: 554–62.
28 Liehn EA, Postea O, Curaj A, Marx N. Repair after myocardial infarction, between fantasy and reality: the role of chemokines. J Am Coll Cardiol 2011;58:2357–62.
29 Ruscher K, Kuric E, Liu Y, Walter HL, Issazadeh-Navikas S, Englund E, et al. Inhibition of CXCL12 signaling attenuates the postischemic immune response and improves functional recovery after stroke. J Cereb Blood Flow Metab 2013. doi: 10.1038/jcbfm.2013.71.
30 Bogoslovsky T, Spatz M, Chaudhry A, Maric D, Luby M, Frank J, et al. Stromal-derived factor-1alpha correlates with circulating endothelial progenitor cells and with acute lesion volume in stroke patients. Stroke 2011;42:618–25.
31 Carbone F, Nencioni A, Mach F, Vuilleumier N, Montecucco F. Pathophysiological role of neutrophils in acute myocardial infarction. Thromb Haemost 2013; doi:10.1160/TH13-03-0211.
32 van Tits LJ, Stienstra R, van Lent PL, Netea MG, Joosten LA, Stalenhoef AF. Oxidized LDL enhances pro-inflammatory responses of alternatively activated M2 macrophages: a crucial role for Kruppel-like factor 2. Atherosclerosis 2011;214:345–9.
33 Liu Y, Hulten LM, Wiklund O. Macrophages isolated from human atherosclerotic plaques produce IL-8, and oxysterols may have a regulatory function for IL-8 production. Arterioscler Thromb Vasc Biol 1997;17: 317–23.
34 Boisvert WA, Santiago R, Curtiss LK, Terkeltaub RA. A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. J Clin Invest 1998;101:353–63.
35 Kukielka GL, Smith CW, LaRosa GJ, Manning AM, Mendoza LH, Daly TJ, et al. Interleukin-8 gene induction in the myocardium after ischemia and reperfusion in vivo. J Clin Invest 1995;95:89–103.
36 Boyle EM, Jr., Kovacich JC, Hebert CA, Canty TG, Jr., Chi E, Morgan EN, et al. Inhibition of interleukin-8 blocks myocardial ischemia-reperfusion injury. J Thorac Cardiovasc Surg 1998;116:114–21.
37 Montecucco F, Lenglet S, Gayet-Ageron A, Bertolotto M, Pelli G, Palombo D, et al. Systemic and intraplaque mediators of inflammation are increased in patients symptomatic for ischemic stroke. Stroke 2010;41: 1394–404.
38 Grau AJ, Reis A, Buggle F, Al-Khalaf A, Werle E, Valois N, et al. Monocyte function and plasma levels of interleukin-8 in acute ischemic stroke. J Neurol Sci 2001;192:41–7.
39 Tarkowski E, Rosengren L, Blomstrand C, Wikkelso C, Jensen C, Ekholm S, et al. Intrathecal release of pro- and anti-inflammatory cytokines during stroke. Clin Exp Immunol 1997;110:492–9.
40 Chandrasekar B, Smith JB, Freeman GL. Ischemia-reperfusion of rat myocardium activates nuclear factor-KappaB and induces neutrophil infiltration via lipopolysaccharide-induced CXC chemokine. Circulation 2001;103:2296-2302.
41 Miyake M, Goodison S, Urquidi V, Gomes Giacoia E, Rosser CJ. Expression of CXCL1 in human endothelial cells induces angiogenesis through the CXCR2 receptor and the ERK1/2 and EGF pathways. Lab Invest 2013;93:768–78.
42 Kocher AA, Schuster MD, Bonaros N, Lietz K, Xiang G, Martens TP, et al. Myocardial homing and neovascularization by human bone marrow angioblasts is regulated by IL-8/Gro CXC chemokines. J Mol Cell Cardiol 2006;40:455–64.
43 Moldobaeva A, Baek A, Eldridge L, Wagner EM. Differential activity of pro-angiogenic CXC chemokines. Microvasc Res 2010;80:18–22.
44 Losy J, Zaremba J, Skrobanski P. CXCL1 (GRO-alpha) chemokine in acute ischaemic stroke patients. Folia Neuropathol 2005;43:97–102.
45 Struyf S, Salogni L, Burdick MD, Vandercappellen J, Gouwy M, Noppen S, et al. Angiostatic and chemotactic activities of the CXC chemokine CXCL4L1 (platelet factor-4 variant) are mediated by CXCR3. Blood 2011;117:480–8.
46 Yates-Binder CC, Rodgers M, Jaynes J, Wells A, Bodnar RJ, Turner T. An IP-10 (CXCL10)-derived peptide inhibits angiogenesis. PLoS One 2012;7:e40812.
47 Bujak M, Dobaczewski M, Gonzalez-Quesada C, Xia Y, Leucker T, Zymek P, et al. Induction of the CXC chemokine interferon-gamma-inducible protein 10 regulates the reparative response following myocardial infarction. Circ Res 2009;105:973–83.
48 Sokolov VO, Krasnikova TL, Prokofieva LV, Kukhtina NB, Arefieva TI. Expression of markers of regulatory CD4+CD25+foxp3+ cells in atherosclerotic plaques of human coronary arteries. Bull Exp Biol Med 2009;147:726–9.
49 Takahashi M. Role of the SDF-1/CXCR4 system in myocardial infarction. Circ J 2010;74:418–23.
50 Schonemeier B, Schulz S, Hoellt V, Stumm R. Enhanced expression of the CXCl12/SDF-1 chemokine receptor CXCR7 after cerebral ischemia in the rat brain. J Neuroimmunol 2008;198:39–45.

51 Penn MS, Pastore J, Miller T, Aras R. SDF-1 in myocardial repair. Gene Ther 2012;19:583–7.
52 Bakondi B, Shimada IS, Peterson BM, Spees JL. SDF-1alpha secreted by human CD133-derived multipotent stromal cells promotes neural progenitor cell survival through CXCR7. Stem Cells Dev 2011;20: 1021–9.
53 Peng H, Wu Y, Duan Z, Ciborowski P, Zheng JC. Proteolytic processing of SDF-1alpha by matrix metalloproteinase-2 impairs CXCR4 signaling and reduces neural progenitor cell migration. Protein Cell 2012; 3:875–82.
54 Liu XL, Zhang PF, Ding SF, Wang Y, Zhang M, Zhao YX, et al. Local gene silencing of monocyte chemoattractant protein-1 prevents vulnerable plaque disruption in apolipoprotein E-knockout mice. PLoS One 2012;7:e33497.
55 Martinovic I, Abegunewardene N, Seul M, Vosseler M, Horstick G, Buerke M, et al. Elevated monocyte chemoattractant protein-1 serum levels in patients at risk for coronary artery disease. Circ J 2005;69: 1484–9.
56 Kumar AG, Ballantyne CM, Michael LH, Kukielka GL, Youker KA, Lindsey ML, et al. Induction of monocyte chemoattractant protein-1 in the small veins of the ischemic and reperfused canine myocardium. Circulation 1997;95:693–700.
57 Hayasaki T, Kaikita K, Okuma T, Yamamoto E, Kuziel WA, Ogawa H, et al. CC chemokine receptor-2 deficiency attenuates oxidative stress and infarct size caused by myocardial ischemia-reperfusion in mice. Circ J 2006;70:342–51.
58 Kaikita K, Hayasaki T, Okuma T, Kuziel WA, Ogawa H, Takeya M. Targeted deletion of CC chemokine receptor 2 attenuates left ventricular remodeling after experimental myocardial infarction. Am J Pathol 2004;165:439–47.
59 Dewald O, Zymek P, Winkelmann K, Koerting A, Ren G, Abou-Khamis T, et al. CCL2/Monocyte Chemoattractant Protein-1 regulates inflammatory responses critical to healing myocardial infarcts. Circ Res 2005; 96:881–9.
60 Haudek SB, Xia Y, Huebener P, Lee JM, Carlson S, Crawford JR, et al. Bone marrow-derived fibroblast precursors mediate ischemic cardiomyopathy in mice. Proc Natl Acad Sci U S A 2006;103:18284–9.
61 Hughes PM, Allegrini PR, Rudin M, Perry VH, Mir AK, Wiessner C. Monocyte chemoattractant protein-1 deficiency is protective in a murine stroke model. J Cereb Blood Flow Metab 2002;22:308–17.
62 Dimitrijevic OB, Stamatovic SM, Keep RF, Andjelkovic AV. Absence of the chemokine receptor CCR2 protects against cerebral ischemia/reperfusion injury in mice. Stroke 2007;38:1345–53.
63 Yang B, Xi X, Aronowski J, Savitz SI. Ischemic stroke may activate bone marrow mononuclear cells to enhance recovery after stroke. Stem Cells Dev 2012;21:3332–40.
64 Yan YP, Sailor KA, Lang BT, Park SW, Vemuganti R, Dempsey RJ. Monocyte chemoattractant protein-1 plays a critical role in neuroblast migration after focal cerebral ischemia. J Cereb Blood Flow Metab 2007;27:1213–24.
65 Shen B, Li J, Gao L, Zhang J, Yang B. Role of CC-chemokine receptor 5 on myocardial ischemia-reperfusion injury in rats. Mol Cell Biochem 2013;378:137–44.
66 Montecucco F, Braunersreuther V, Lenglet S, Delattre BM, Pelli G, Buatois V, et al. CC chemokine CCL5 plays a central role impacting infarct size and post-infarction heart failure in mice. Eur Heart J 2012;33: 1964–74.
67 Terao S, Yilmaz G, Stokes KY, Russell J, Ishikawa M, Kawase T, et al. Blood cell-derived RANTES mediates cerebral microvascular dysfunction, inflammation, and tissue injury after focal ischemia-reperfusion. Stroke 2008;39:2560–70.
68 Tokami H, Ago T, Sugimori H, Kuroda J, Awano H, Suzuki K, et al. RANTES has a potential to play a neuroprotective role in an autocrine/paracrine manner after ischemic stroke. Brain Res 2013;1517:122–32.
69 Proulx C, El-Helou V, Gosselin H, Clement R, Gillis MA, Villeneuve L, et al. Antagonism of stromal cell-derived factor-1alpha reduces infarct size and improves ventricular function after myocardial infarction. Pflugers Arch 2007;455:241–50.
70 Morimoto H, Takahashi M, Shiba Y, Izawa A, Ise H, Hongo M, et al. Bone marrow-derived CXCR4+ cells mobilized by macrophage colony-stimulating factor participate in the reduction of infarct area and improvement of cardiac remodeling after myocardial infarction in mice. Am J Pathol 2007;171:755–66.
71 Jujo K, Hamada H, Iwakura A, Thorne T, Sekiguchi H, Clarke T, et al. CXCR4 blockade augments bone marrow progenitor cell recruitment to the neovasculature and reduces mortality after myocardial infarction. Proc Natl Acad Sci U S A 2010;107:11008–13.
72 Dai S, Yuan F, Mu J, Li C, Chen N, Guo S, et al. Chronic AMD3100 antagonism of SDF-1alpha-CXCR4 exacerbates cardiac dysfunction and remodeling after myocardial infarction. J Mol Cell Cardiol 2010;49: 587–97.
73 Huang C, Gu H, Zhang W, Manukyan MC, Shou W, Wang M. SDF-1/CXCR4 mediates acute protection of cardiac function through myocardial STAT3 signaling following global ischemia/reperfusion injury. Am J Physiol Heart Circ Physiol 2011;301:H1496–505.
74 Jujo K, Ii M, Sekiguchi H, Klyachko E, Misener S, Tanaka T, et al. CXC-chemokine receptor 4 antagonist AMD3100 promotes cardiac functional recovery after ischemia/reperfusion injury via endothelial nitric oxide synthase-dependent mechanism. Circulation 2013;127:63–73.
75 Luo Y, Zhao X, Zhou X, Ji W, Zhang L, Luo T, et al. Short-term intermittent administration of CXCR4 antagonist AMD3100 facilitates myocardial repair in experimental myocardial infarction. Acta Biochim Biophys Sin (Shanghai) 2013;45:561–69.
76 Huang J, Li Y, Tang Y, Tang G, Yang GY, Wang Y. CXCR4 antagonist AMD3100 protects blood-brain barrier integrity and reduces inflammatory response after focal ischemia in mice. Stroke 2013;44:190–97.
77 Garau A, Bertini R, Colotta F, Casilli F, Bigini P, Cagnotto A, et al. Neuroprotection with the CXCL8 inhibitor repertaxin in transient brain ischemia. Cytokine 2005;30:125–31.
78 Villa P, Triulzi S, Cavalieri B, Di Bitondo R, Bertini R, Barbera S, et al. The interleukin-8 (IL-8/CXCL8) receptor inhibitor reparixin improves neurological deficits and reduces long-term inflammation in permanent and transient cerebral ischemia in rats. Mol Med 2007;13:125–33.
79 Sousa LF, Coelho FM, Rodrigues DH, Campos AC, Barcelos Lda S, Teixeira MM, et al. Blockade of CXCR1/2 chemokine receptors protects against brain damage in ischemic stroke in mice. Clinics (Sao Paulo) 2013;68:391–4.
80 Kanki S, Segers VF, Wu W, Kakkar R, Gannon J, Sys SU, et al. Stromal cell-derived factor-1 retention and cardioprotection for ischemic myocardium. Circ Heart Fail 2011;4:509–18.
81 Jang YH, Kim JH, Ban C, Ahn K, Cheong JH, Kim HH, et al. Stromal cell derived factor-1 (SDF-1) targeting reperfusion reduces myocardial infarction in isolated rat hearts. Cardiovasc Ther 2012;30:264–72.
82 Braunersreuther V, Pellieux C, Pelli G, Burger F, Steffens S, Montessuit C, et al. Chemokine CCL5/RANTES inhibition reduces myocardial reperfusion injury in atherosclerotic mice. J Mol Cell Cardiol 2010;48: 789–98.
83 Montecucco F, Lenglet S, Braunersreuther V, Pelli G, Pellieux C, Montessuit C, et al. Single administration of the CXC chemokine-binding protein Evasin-3 during ischemia prevents myocardial reperfusion injury in mice. Arterioscler Thromb Vasc Biol 2010;30:1371–77.
84 Copin JC, da Silva RF, Fraga-Silva RA, Capettini L, Quintao S, Lenglet S, et al. Treatment with Evasin-3 reduces atherosclerotic vulnerability for ischemic stroke, but not brain injury in mice. J Cereb Blood Flow Metab 2013;33:490–8.
85 Oral H, Kanzler I, Tuchscheerer N, Curaj A, Simsekyilmaz S, Sonmez TT, et al. CXC chemokine KC fails to induce neutrophil infiltration and neoangiogenesis in a mouse model of myocardial infarction. J Mol Cell Cardiol 2013;60:1–7.
86 Liehn EA, Piccinini AM, Koenen RR, Soehnlein O, Adage T, Fatu R, et al. A new monocyte chemotactic protein-1/chemokine CC motif ligand-2 competitor limiting neointima formation and myocardial ischemia/reperfusion injury in mice. J Am Coll Cardiol 2010;56:1847–57.
87 Braunersreuther V, Steffens S, Arnaud C, Pelli G, Burger F, Proudfoot A, et al. A novel RANTES antagonist prevents progression of established atherosclerotic lesions in mice. Arterioscler Thromb Vasc Biol 2008; 28:1090–96.
88 Jantunen E. Novel strategies for blood stem cell mobilization: special focus on plerixafor. Expert Opin Biol Ther 2011;11:1241–48.
89 Deruaz M, Frauenschuh A, Alessandri AL, Dias JM, Coelho FM, Russo RC, et al. Ticks produce highly selective chemokine binding proteins with antiinflammatory activity. J Exp Med 2008;205:2019–31.
90 Di Filippo C, Rossi F, Rossi S, D'Amico M. Cannabinoid CB2 receptor activation reduces mouse myocardial ischemia-reperfusion injury: involvement of cytokine/chemokines and PMN. J Leukoc Biol 2004;75: 453–9.

91 Montecucco F, Lenglet S, Braunersreuther V, Burger F, Pelli G, Bertolotto M, et al. CB(2) cannabinoid receptor activation is cardioprotective in a mouse model of ischemia/reperfusion. J Mol Cell Cardiol 2009;46:612–20.
92 Murikinati S, Juttler E, Keinert T, Ridder DA, Muhammad S, Waibler Z, et al. Activation of cannabinoid 2 receptors protects against cerebral ischemia by inhibiting neutrophil recruitment. Faseb J 2010; 24:788–98.
93 Fernandez-Lopez D, Faustino J, Derugin N, Wendland M, Lizasoain I, Moro MA, et al. Reduced infarct size and accumulation of microglia in rats treated with WIN 55,212-2 after neonatal stroke. Neuroscience 2012;207:307–15.
94 Huber BC, Brunner S, Segeth A, Nathan P, Fischer R, Zaruba MM, et al. Parathyroid hormone is a DPP-IV inhibitor and increases SDF-1-driven homing of CXCR4(+) stem cells into the ischaemic heart. Cardiovasc Res 2011;90:529–37.
95 Zhang B, Subramanian S, Dziennis S, Jia J, Uchida M, Akiyoshi K, et al. Estradiol and G1 reduce infarct size and improve immunosuppression after experimental stroke. J Immunol 2010;184:4087–94.
96 Chen Y, Jin X, Zeng Z, Liu W, Wang B, Wang H. Estrogen-replacement therapy promotes angiogenesis after acute myocardial infarction by enhancing SDF-1 and estrogen receptor expression. Microvasc Res 2009;77:71–7.
97 Chen Y, Fu L, Han Y, Teng Y, Sun J, Xie R, et al. Testosterone replacement therapy promotes angiogenesis after acute myocardial infarction by enhancing expression of cytokines HIF-1a, SDF-1a and VEGF. Eur J Pharmacol 2012;684:116–24.
98 Sironi L, Banfi C, Brioschi M, Gelosa P, Guerrini U, Nobili E, et al. Activation of NF-kB and ERK1/2 after permanent focal ischemia is abolished by simvastatin treatment. Neurobiol Dis 2006;22:445–51.
99 Iwata A, Miura S, Shirai K, Kawamura A, Tomita S, Matsuo Y, et al. Lower level of low-density lipoprotein cholesterol by statin prevents progression of coronary restenosis after successful stenting in acute myocardial infarction. Intern Med 2006;45:885–90.
100 Cui X, Chopp M, Zacharek A, Roberts C, Lu M, Savant-Bhonsale S, et al. Chemokine, vascular and therapeutic effects of combination Simvastatin and BMSC treatment of stroke. Neurobiol Dis 2009;36: 35–41.
101 Qiu R, Cai A, Dong Y, Zhou Y, Yu D, Huang Y, et al. SDF-1alpha upregulation by atorvastatin in rats with acute myocardial infarction via nitric oxide production confers anti-inflammatory and anti-apoptotic effects. J Biomed Sci 2012;19:99.
102 Wayman NS, Hattori Y, McDonald MC, Mota-Filipe H, Cuzzocrea S, Pisano B, et al. Ligands of the peroxisome proliferator-activated receptors (PPAR-gamma and PPAR-alpha) reduce myocardial infarct size. Faseb J 2002;16:1027–40.
103 Ito H, Nakano A, Kinoshita M, Matsumori A. Pioglitazone, a peroxisome proliferator-activated receptor-gamma agonist, attenuates myocardial ischemia/reperfusion injury in a rat model. Lab Invest 2003; 83:1715–21.
104 Nakamura Y, Yamada Y, Shimomura H, Nagayoshi Y, Tsujita K, Yamashita T, et al. Effect of edaravone on plasma monocyte chemoattractant protein-1 levels in patients with acute myocardial infarction. J Cardiol 2009;54:416–24.
105 Montecucco F, Bauer I, Braunersreuther V, Bruzzone S, Akhmedov A, Luscher TF, et al. Inhibition of nicotinamide phosphoribosyltransferase reduces neutrophil-mediated injury in myocardial infarction. Antioxid Redox Signal 2013;18:630–41.
106 Horuk R. Chemokine receptor antagonists: overcoming developmental hurdles. Nat Rev Drug Discov 2009;8:23–33.
107 Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. Nat Immunol 2006;7:311–7.
108 Sorensen LN, Paludan SR. Blocking CC chemokine receptor (CCR) 1 and CCR5 during herpes simplex virus type 2 infection in vivo impairs host defence and perturbs the cytokine response. Scand J Immunol 2004;59:321–33.
109 Wallis RS. Infectious complications of tumor necrosis factor blockade. Curr Opin Infect Dis 2009;22:403–9.
110 Lionakis MS, Kontoyiannis DP. Glucocorticoids and invasive fungal infections. Lancet 2003;362:1828–38.