Biomedicine & Pharmacotherapy 139 (2021) 111558

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Review

Potential anti-inflammatory effect of erythropoietin in non-clinical studies in vivo: A systematic review

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ARTICLE INFO

Keywords:
Erythropoietin
Inflammation
Non-clinical studies in vivo
Animal models
Rodents

ABSTRACT

Erythropoietin (EPO) is a hypoxia-induced hormone produced in adult kidneys with erythropoietic and non-erythropoietic effects. In vivo studies represent an important role to comprehend the efficacy and safety in the early phase of repurposing drugs. The aim is to evaluate the potential anti-inflammatory effect of EPO observed in animal models of disease. Following PRISMA statements, electronic database Medline via PubMed platform was used to search articles with the research expression ((erythropoietin [MeSH Terms]) AND (inflammation [MeSH Terms]) AND (disease models, animal [MeSH Terms])). The inclusion criteria were original articles, studies where EPO was administered, studies where inflammation was studied and/or evaluated, non-clinical studies in vivo with rodents, and articles published in English. Thirty-six articles met the criteria for qualitative analysis. Exogenous EPO was used in models of sepsis, traumatic brain injury, and autoimmune neuritis, with an average of 3000 IU/Kg for single and multiple doses, using mice and rats. Biomarkers such as immune-related effectors, cytokines, reactive oxygen species, prostaglandins, and other biomarkers were assessed. EPO has been recognized as a multifunctional cytokine with anti-inflammatory properties, showing its significant effect both in acute and chronic models of inflammation. Further non-clinical studies are suggested for the enlightenment of anti-inflammatory mechanisms of EPO in lower doses, allowing us to understand the translational data for humans.

1. Introduction

Erythropoietin (EPO) is an endogenous glycoprotein hormone that regulates the production, survival, and differentiation of red blood cells [1–4]. This molecule with 30,4 kDa is mainly produced by the fetal liver and adult kidney by hypoxia stimulus [1,2,5,6]. As a hypoxia-induced hormone, engages the expression of hypoxia-inducible factor (HIF) which regulates transcription of the EPO gene in kidneys [2,6–8]. The EPO mechanism of action is triggered when EPO receptor (EPOR) is activated by EPO through the cleavage of GATA-binding factor 1, inducing activation of Janus kinase(JAK)−2 [3,6,9–13]. EPO is commonly used for the treatment of anemia associated with chronic kidney disease in humans, but also reveals non-erythropoietic protective functions such as the inhibition of apoptosis, autophagy, induction of angiogenesis, anti-inflammatory effect, neuroprotection, and tissue regeneration [2,3,6,9]. Despite this, EPO presents high side effects due to erythropoiesis stimulation and leads to the risk of severe cardiovascular events [3,5]. In this sense, it is important to modify the EPO molecule, maintaining similar pharmacokinetics characteristics to EPO, although doesn't binding to the conventional homodimeric EPOR, lacking erythropoietic side effects. Thus it is possible to maintain efficacy and improve the safety of EPO.

Inflammatory diseases are globally identified as one of the major causes of morbidity and mortality across the population [14,15]. Inflammation is the basis of many diseases, leading to a life-long debilitating illness, with high costs for therapy [16]. Current therapies

Abbreviations: cEPO, carbamylated erythropoietin; EPO, erythropoietin; EPOR, erythropoietin recptor; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; JAK, Janus Kinase; rHuEPO, recombinant human erythropoietin; TGF, transforming growth factor; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TNF, tumor necrosis factor.

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https://doi.org/10.1016/j.biopha.2021.111558
Received 21 December 2020; Received in revised form 19 February 2021; Accepted 23 March 2021
Available online 22 April 2021

are limited to steroidal, non-steroidal, and biological anti-inflammatory agents to suppress inflammation [17]. Chronic use of these therapies for inflammatory diseases leads to some serious adverse effects like gastrointestinal, renal, respiratory, cardiovascular damages, and reduction in host defense against infections [14,17]. Thus, it's crucial to find new pharmacological approaches with selective action that could revert the mechanisms of inflammation with lesser toxicity [14,18,19].

Our research group has developed previous non-clinical studies in an acute model of 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis, testing EPO with beneficial effects in the progression and treatment of Inflammatory Bowel Disease (IBD) [20]. From this point of view, the present review intended to focus on one non-erythropoietic effect of erythropoietin such as its role in the inflammatory response [14]. Hence, this systematic review aims to describe the potential anti-inflammatory effect of erythropoietin in non-clinical studies in vivo with rodents.

As expected, EPO has been recognized as a multifunctional cytokine with anti-inflammatory properties therefore, this molecule can be an interesting candidate to treat inflammatory-based diseases, improving patient's quality of life.

2. Material and methods

2.1. Search strategy

Following the PRISMA statements, the electronic database Medline via PubMed platform was used for searches carried out in April 2020 to identify articles of interest. A research strategy was developed, and combinations of keywords ("erythropoietin", "inflammation" and "disease models, animal") were performed through MeSH terms obtained at MeSH Database, leading to the following search string used: ((erythropoietin [MeSH Terms]) AND (inflammation [MeSH Terms]) AND (disease models, animal [MeSH Terms])).

2.2. Selection of studies

The included articles from the search string were selected according to the following inclusion criteria: only original articles, studies with animal models where EPO was administered, studies where inflammation was studied and/or evaluated, non-clinical studies in vivo with rodents, and articles published in English. The exclusion criteria were review articles, expert opinions, book chapters, studies with only in vitro procedures, and studies where EPO is used for the treatment of diseases included in the summary of product characteristics (SPC).

2.3. Eligibility of articles

The results obtained were identified by two independent reviewers, on the same day at the same time. Once the inclusion and exclusion criteria have been established and the search string applied, the full text of the articles has been read to determine their eligibility for inclusion or exclusion. Disagreements between reviewers were resolved by mutual consensus.

2.4. Data extraction

The table of results is organized based on erythropoietin-related parameters (EPO type, dose, frequency and duration, route of administration, and disease animal model); animal-related parameters (animal, strain, gender, and age), and ultimately, biomarkers assessed. Thereby, information was extracted and evaluated.

3. Results and discussion

Through the search expression, in the electronic database, a total of 62 articles were obtained. These articles were screened according to the inclusion and exclusion criteria. Thereafter, 26 articles were excluded, and no duplicates were identified. The reasons for exclusion were: studies with no EPO administration (n = 10), studies where EPO is already used for the treatment of diseases included in the SPC (n = 8), no evaluation of the inflammatory process or response (n = 2), review articles (n = 2), in vitro article only (n = 1), opinion article (n = 1), book chapter (n = 1), and full text not available (n = 1). Thus, 36 original articles were included in the qualitative analysis of this systematic review. The results of the literature obtained through search expression are observed in Fig. 1.

The following table was designed to extract relevant information, namely administrations of erythropoietin in animal models of rodents and its effects on the inflammatory response through biomarkers used. This will allow understanding which type of EPO was used, the dose, frequency and duration, and which routes of administration were used. The different animal models of disease will help to understand the type of rodent used, its strain, gender, and age. Finally, biomarkers were evaluated to conclude if the EPO has anti-inflammatory activity.

Thus, the results obtained through the analysis of the articles are shown in Table 1.

3.1. Erythropoietin-related parameters

3.1.1. EPO type

Although all EPO used is exogenous recombinant erythropoietin, some articles discriminate their type. Most articles use only the term rHuEPO (n = 22), others mention epoetin-α (n = 8), epoetin-β (n = 3). Yet, ARA 290 (n = 2), carbamylated erythropoietin (cEPO) (n = 2) and darbepoetin-α (n = 1) are mentioned. Since most articles do not specify the type of EPO used, it becomes difficult to assume which is the most used and why. By definition, considering that all types of EPO used are exogenous only the term EPO, adopted by many researchers, will be used throughout this review.

All recombinant human erythropoietin (rHuEPO) has demonstrated anti-inflammatory properties with a reduction of the assessed inflammatory biomarkers, such as tumor necrosis factor (TNF)-α, interleukin (IL)−1β, and IL-6 [21,22,54,55].

rHuEPO agents as epoetin-α, epoetin-β, epoetin-ɣ, epoetin-ω, epoetin-δ, and darbepoetin-α exhibit the same polypeptide chain of 165 amino acids, identical to endogenous EPO, but differs in carbohydrate structure. The greek letters indicate differences in the glycosylation pattern but with the same amino acid sequence and therefore, different serum half-life [56]. Glycosylation is important for the biologic activity of EPO analogs and an alteration in the glycan chains promotes different in vivo and in vitro activity [57]. In the endogenous EPO, most sialic acid residues are attached to the three N-linked glycosylation chains with a diversity of functions such as the protection of EPO molecule from proteases and the modulation of its receptor binding affinity [57].

Epoetin-α, epoetin-β, and darbepoetin-α may have been used since they were already commercialized and two of them have a longer half-life than endogenous EPO (5 h). Since the half-life of epoetin-β (8,8 h) is longer than epoetin-α (4,5 h), lower doses may be needed to maintain hemoglobin and hematocrit in the target level [58]. Darbepoetin-α confers a clinical advantage over epoetin's due to its increased half-life (25,3 h) by allowing less frequent dosing while safely. However, it is more expensive than epoetin's [59]. Darbepoetin-α is a hyperglycosylated molecule that stimulates erythropoiesis by the same mechanism as the endogenous EPO [10]. Although all EPO-derivates are highly similar to endogenous EPO, an increase in the number of glycosylation sites in the carbohydrate structure enhances rHuEPO activity [59]. This rHuEPO agent has a longer elimination half-life, since presents two additional N-glycans chains than other epoetin's [59].

Additionally, the use of the cEPO or ARA 290 can be an advantage, since they preserve the EPO efficacy combined with a better safety profile (Fig. 2). cEPO is a non-erythropoietic erythropoietin, because it is a carbamoyl derivative of the EPO modification [60,61]. The cEPO results from the carbamylation of EPO lysine residues, in which a

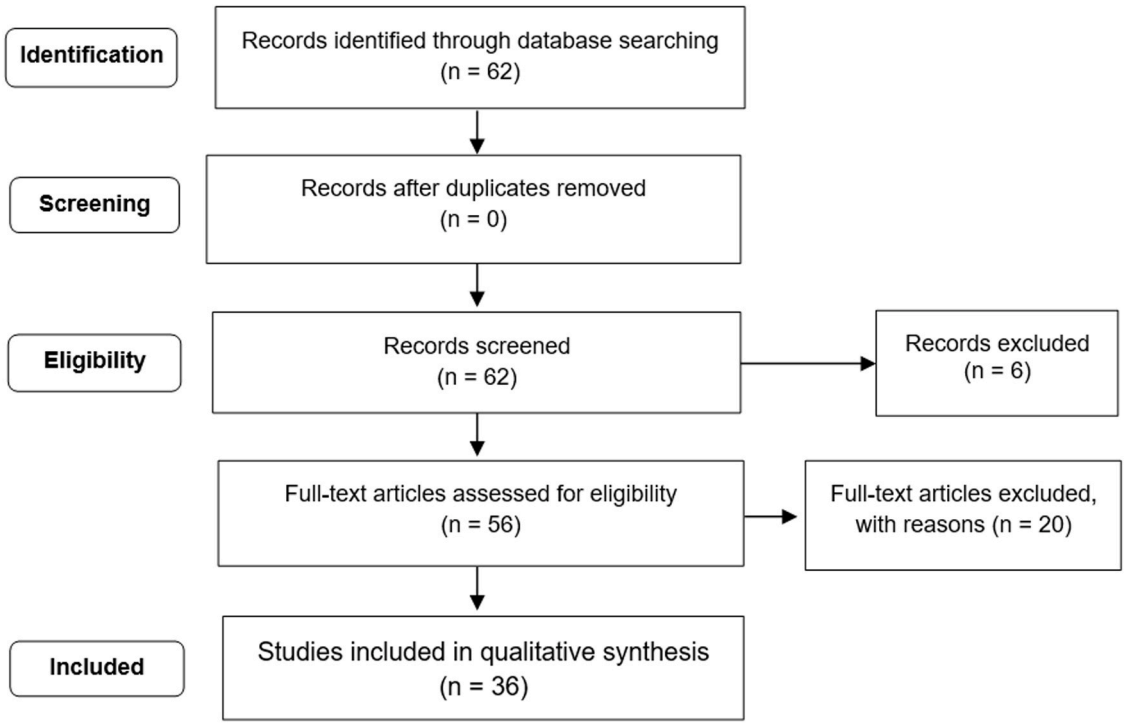

Fig. 1. PRISMA flow diagram with the results of the literature.

carbamyl group is irreversibly coupled to each of the residues [61]. This functional change is achieved through the N-terminal alanine in the EPO polypeptide chain with eight lysine residues, providing nine amino groups for carbamylation [62]. cEPO presents similar pharmacokinetics characteristics to EPO although, binds to heterodimeric EPO common-β receptor complex (CD131), lacking erythropoietic side effects [12,60,61,63–65]. ARA 290 is a pyroglutamate molecule with a molecular weight of 1257 kDa. This molecule was modeled from the three-dimensional structure of helix B of the EPO molecule [11,66,67]. ARA 290 is responsible to exert the selective tissue-protective and this way does not show any hematopoietic activity, since selectively binds the heteromeric EPO-CD131 complex, eliminating its side effects [11,66–69].

3.1.2. Dose

In these studies, the most commonly used EPO doses were 1000 (n = 9), 3000 (n = 6), and 5000 (n = 15) IU/Kg (Fig. 3). Considering all the doses used, the average of single and multiple doses was 3000 IU/Kg and the mode was 5000 IU/Kg.

Through prior studies conducted by Jin et al. [72] and Cerri et al. [70], it was possible to define that the use of doses of 3000 and 5000 IU/Kg has achieved a reduction of inflammation and therefore, may be the reason why many researchers have applied these doses [70–73]. In a rat model of sepsis, Souza et al. [74] reported that pre or post-treatment with EPO (4000 IU/kg) exhibited anti-inflammatory effects such as the inhibition of factor nuclear kappa B (NF-κB) pathway activation, reduction of proinflammatory cytokines, and also improved survival. This is in accordance with the results obtained [74]. When a lower EPO dose is used, the observed findings are more controversial. Mori et al. [75] demonstrated that in a model of intestinal ischemia-reperfusion a single SC dose of 1000 IU/Kg of EPO provided a reduction in inflammatory cytokines [48,75]. However, in a model of endotoxic stroke with four doses, Aoshiba et al. [27] also used 1000 IU/Kg of EPO and there was only observed a reduction in the mortality rate, although without apparently alterations in the inflammatory response [27].

In humans, the EPO maximum recommended dose is 1050 IU/kg/week (80.000 UI for an adult with normal weight). Since the mice or rat weights are between 25 and 40 g and 300–500 g, respectively, the administration of EPO doses around 30 UI/week or 400 UI/week would be recommended based on humans guidelines. Despite the EPO dose of 5000 IU/Kg is the option most used in the non-clinical studies in vivo to evaluate its anti-inflammatory effect in a context of proof of content, the studies using a lower EPO dose with significant anti-inflammatory outcomes are crucial in order to allow correlating the translational data to humans.

3.1.3. Frequency and duration of administrations

Most studies used a single dose of EPO (n = 15). Other studies promote treatment with twice administrations of EPO (n = 6). In the studies where EPO daily doses are used (n = 11), the duration of treatment varied from 3 days to 3 months. The frequency and duration of EPO administrations are related to acute and/or chronic treatment whereupon one study considered an acute treatment when a single administration is made and chronic treatment with a daily administration for more than 4 days [22].

All studies, both acute and chronic, where EPO was used demonstrated benefic results with anti-inflammatory effects [76]. During chronic treatment, EPO also prevented mortality rate [22]. The frequency and duration of EPO administration influence its efficacy, but also its safety. This factor is so important that some studies designed the EPO treatment once every 2 weeks trying to avoid erythropoietic effects [54,77]. The risk of adverse effects with the EPO treatment, dependently on increased hematocrit, is higher from day 8 [78]. Parsa et al. [77] even found that side effects take place after a minimum of 4 days of EPO treatment [54,77]. In our acute model of TNBS-induced colitis, hematocrit was not affected by EPO administration [20]. Similar values of hematocrit were detected between EPO treated mice and non-colitic mice [35,79].

Several studies showed that the use of endogenous EPO leads to the risk of severe cardiovascular events increasing the incidence of thromboembolism, hypertension, and tumor progression [5,56,80]. The innovative approach with cEPO treatment, instead of the rHuEPO, may minimize the risk of these potential adverse side effects [62]. After the carbamylation process, cEPO preserves the three-dimensional structure

Table 1
Outcomes of potential anti-inflammatory effect of erythropoietin in non-clinical studies in vivo.

Erythropoietin-Related Parameters					Animal-Related Parameters				Biomarkers Assessed	Reference
EPO type	**Dose (IU/Kg)**	**Frequency / Duration**	**Route of Administration**	**Disease Animal Model**	**Animal**	**Strain**	**Gender**	**Age (weeks)**		
rHuEPO	5	Daily, 7days	IC	Sepsis	Rats	Sprague Dawley	Male	–	JAK2/STAT3	[21]
rHuEPO	300, 1200	Single dose; daily, 4days	SC	Sepsis	Rats	Wistar	Male	12–16	CAT, SOD	[22]
	1000	Single dose	IP	Myocardial injury	Mice / Rats	Kunming, Sprague Dawley	Male	6–8	TNF-α, IL-6, IL-1β, IL-10, NF-κB, CRP	[23]
		Single dose	IV	Uterus inflammation	Rats	Albino Wistar	Female	–	Neutrophils	[24]
		3 doses	SC	Acute esophageal burn	Rats	Sprague Dawley	Female	–	NO, CAT, SOD	[25]
		Single dose	SC	Sepsis	Mice	Swiss Albino	Male	12–16	NO, iNOS, eNOS	[26]
		4 doses	SC	Endotoxic stroke	Mice	BALB/c	Male	7	TNF-α, IL-6, IL-1β, iNOS	[27]
	2500	Twice, 3 days	–	Traumatic brain Injury	Rats	Sprague Dawley	Male	12	TNF-α, IL-1β, IFN-ɣ, IL-4, IL-5, IL-6, IL-10, IL-13	[28]
		Single dose	IP	Ischemic brain injury	Mice	C57BL/6	–	12–72	Microglia cells	[29]
	Erythropoietin-Related Parameters				**Animal-Related Parameters**				**Biomarkers Assessed**	**Reference**
	Disease Model	**Frequency / Duration**	**Route of Administration**	**Disease Animal Model**	**Animal**	**Strain**	**Gender**	**Age (weeks)**		
	3000	Single dose	SC	Obstructive jaundice	Rats	Albino Wistar	–	–	TNF-α, IL-6, IL-1β, CRP	[30]
	3750	Twice	IP	Sepsis	Rats	Sprague Dawley	Male	6–8	T cells	[31]
	3000, 4000, 5000	3 – 7 doses	IP	Spinal cord compressive injury	Rats	Sprague Dawley	Male	12–16	Macrophages	[32]
	5000	Single dose	IP	Endotoxic stoke	Rats	Wistar	Male	3	TNF-α, IL-1β, IL-6	[33]
		Daily, 3 days	IP	Traumatic brain injury	Mice	C57BL/6	Male	12–16	T cells, neutrophils, microglia cells, IL-10, IL-1β, TNF-α, TGF-β	[34]
		Twice	–	Traumatic brain injury	Mice	Sabra	Male	8–12	Microglia cells	[35]
		Twice	–	Brain injury	Mice	BALB/c	–	< 1	IL-6, IL-1β, NF-κB, iNOS, HIF-1α	[36]
		Twice	IP	Intracerebral hemorrhage model	Rats	Wistar	–	1	TNF-α, IL-6, IL-1β	[37]
		Single dose	IV	Ischemia—reperfusion injury	Rats	Lewis	Male	–	NO, eNOS, IL-6	[38]
		Daily, 14 days	IP	Autoimmune neuritis	Rats	Lewis	Male	8–10	T cells, macrophages, HIF-1α	[39]
	Erythropoietin-Related Parameters				**Animal-Related Parameters**				**Biomarkers Assessed**	**Reference**
	Dose (IU/Kg)	**Frequency / Duration**	**Route**	**Disease Model**	**Animal**	**Strain**	**Gender**	**Age (weeks)**		
rHuEPO	5000	Single dose; 3xday, 3 days	SC	Peripheral inflammatory pain	Rats	–	–	< 1	TNF-α, IL-6, IL-1β	[40]
	100, 250, 500	1xweek, 60 days	IP	Amyotrophic lateral sclerosis	Mice	Transgenic	–	8	COX-2, PGE2, microglia cells	[41]
Epoetin-α	500, 1000	4 days	IP	Acute colitis	Mice	CD-1	Male	6–10	Colon lenght, MPO, TNF-α, IL-1β, IL-10	[20]
	1000, 5000	Daily, 3 days	IV	Autoimmune encephalomyelitis	Mice	C57BL/6	Female	8–10	Macrophages, T cells, TNF-α, IFN-ɣ, IL-1β, IL-2, IL-4, IL-5, IL-10, IL-17	[42]
	3000	Twice	SC	Acute kidney and acute lung injury	Mice	Balb/c	Male	6–8	VEGF	[43]
		Daily, 6 days	IP	Lung injury	Rats	Sprague Dawley	–	< 1	TNF-α, MPO	[44]
		Twice	IP	Testicular torsion	Rats	Sprague Dawley	Male	12–16	TNF-α, IL-6, IL-1β, NO	[45]
	5000	Single dose	IP	Autoimmune neuritis	Rats	Lewis	Female	8	Macrophages, T cells, IL-10, IFN-ɣ TGF-β	[46]

(*continued on next page*)

Table 1 (*continued*)

		Daily, 7 days	IP	Malaria	Mice	C57BL/6	Female	5	HIF-1α	[47]
		Erythropoietin-Related Parameters				**Animal-Related Parameters**			**Biomarkers**	**Reference**
	Dose (IU/Kg)	**Frequency / Duration**	**Route**	**Disease Model**	**Animal**	**Strain**	**Gender**	**Age (weeks)**	**Assessed**	
Epoetin-β	2000	Single dose	IP	Sepsis	Rats	Sprague Dawley	Male	8	TNF-α, IL-1β	[48]
	3000	Single dose	IP	Endotoxemia	Rats	Sprague Dawley	Male	–	TNF-α, IL-6, IL-1β, CRP, neutrophils	[49]
	5000	Daily, 3 days	IP	Malaria	Mice	CBA/J	Female	6–8	TNF-α, IFN-ɣ, NO	[50]
Darbepoeti-α	1000	Single dose	IP	Cholestatic fibrosis	Mice	C57BL/6 J	Male	8–10	Macrophages, TGF-β, HIF-1α	[51]
ARA 290	–	Daily, 14 days	IP	Autoimmune neuritis	Rats	Lewis	Male	8–10	Macrophages, T cells, TNF-α, IL-10, TGF-β, iNOS	[52]
	–	Daily, 3 months	IP	Systemic lupus erythematosus	Mice	C57BL/6, MRL/lpr	–	10–12	Macrophages, TNF-α, IL-6, IL-10, TGF-β, IFN-ɣ	[53]
rHuEPO+cEPO	1000	Single dose	IV	Brain death	Rats	Fisher F344	Male	12–16	IL-1β, IL-6, VCAM-1	[54]
		Erythropoietin-Related Parameters				**Animal-Related Parameters**			**Biomarkers**	**Reference**
rHuEPO+cEPO	**Dose (IU/Kg)**	**Frequency / Duration**	**Route**	**Disease Model**	**Animal**	**Strain**	**Gender**	**Age (weeks)**	**Assessed**	
	5000	Single dose	IP	Periventricular leukomalacia	Mice	C57BL/6	–	–	Microglia cells	[55]

Legend: CAT - catalase; cEPO – carbamylated erythropoietin; COX-2 – cyclooxygenase 2; CRP – c-reactive protein; eNOS – endothelial nitric oxide synthase; HIF-1α - hypoxia -inducible factor 1-alpha; IC – Intracardiac; IFN-ɣ - interferon gamma; IL – interleukin; iNOS - inducible nitric oxide synthase; IP – intraperitoneal; IV - intravenous; JAK2/STAT3 – Janus Kinase-2, Signal transducer and activator of transcription-3; MPO – myeloperoxidase; NF-κB – factor nuclear kappa B; NO – nitric oxide; PGE2 – prostaglandin E2; rHuEPO - recombinant human erythropoietin; SC – subcutaneous; SOD – superoxide dismutase; TGF-β - transforming growth factor beta; TNF-α - tumor necrosis factor alpha; VCAM-1 - vascular cell adhesion molecule 1; VEGF – vascular endothelial growth factor.

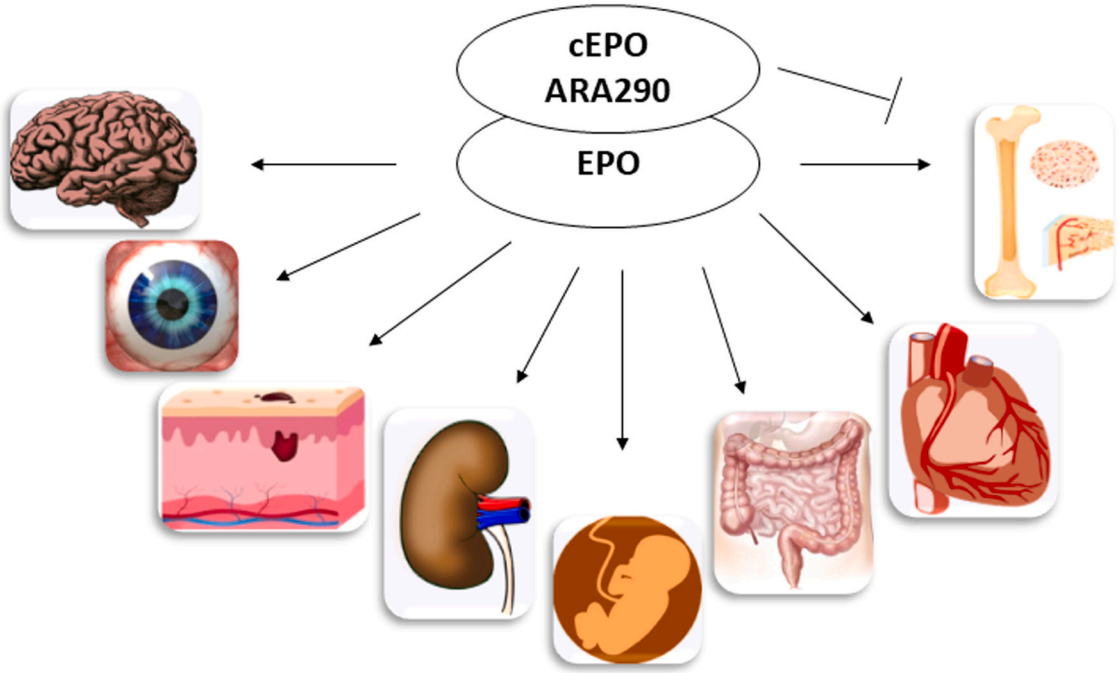

Fig. 2. Mechanism of action of EPO, *cEPO and ARA290* .

of endogenous EPO. However, cEPO effects become mediated by binding to a heterodimeric EPO common-β receptor (CD131) (also shared by GM-CSF, IL-3, and IL-5), while endogenous EPO effects are mediated by binding to a homodimeric EPOR [82] (Fig. 4). The heteroreceptor activation, composed of EPOR and CD131, promotes a protective response through an anti-inflammatory effect, similar to those produced by endogenous EPO [60]. This functional change in its mechanism of action promotes the inhibition of erythropoiesis, maintaining the anti-inflammatory effect. Thus, cEPO gained focus in recent years, since it provides protective properties and anti-inflammatory effect in many diseases, with no hematopoietic or vasoconstrictive activity associated [62,81].

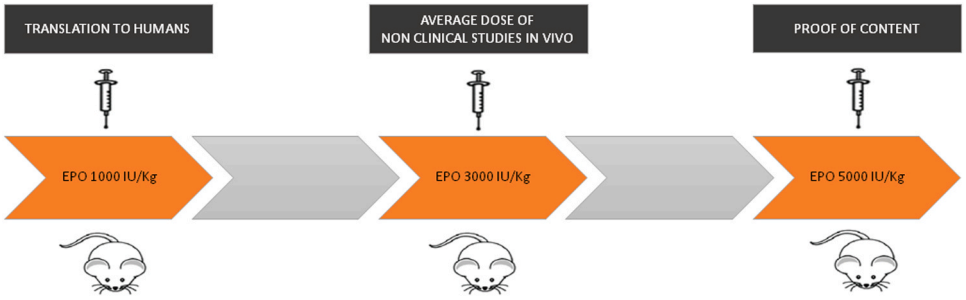

Fig. 3. Scheme of EPO doses used to depend on the purpose of the study.

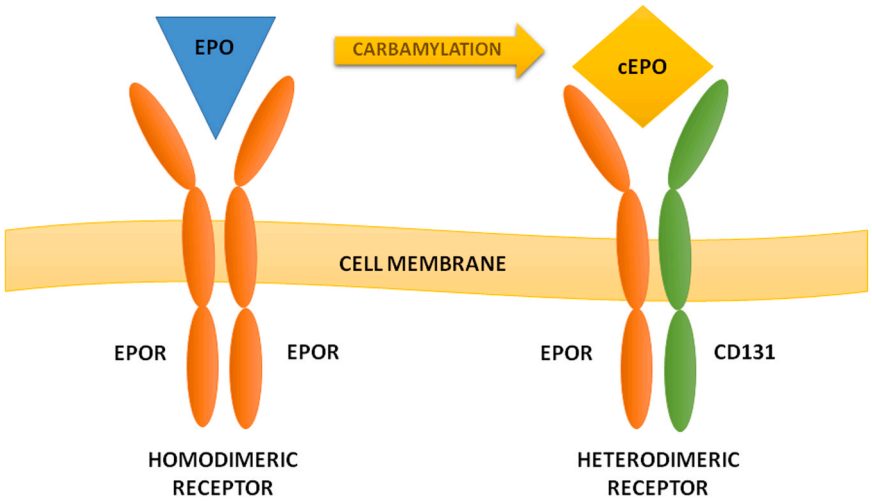

Fig. 4. Mechanism of action of the *cEPO* after carbamylation process.

3.1.4. Route of administration

The route chosen for drug administration depends upon the species, volume, and material to be injected. In these studies, three major routes of EPO administration were used in the analyzed articles, namely the intraperitoneal (n = 19), the subcutaneous (n = 7), and the intravenous (n = 4).

The intraperitoneal route for EPO administration may be preferred. Intraperitoneal administration repeated daily, for up to one month, is well-tolerated in rodents, allows easy handling, and is used for small species. In addition, intraperitoneal administration results in faster absorption than subcutaneous administration and can be used to administer large volumes of fluid safely [79]. Intraperitoneal and subcutaneous administrations can also be given early, which can be an advantage in studies that use newborns [79]. The intravenous route is mainly intended for rats. However, one study used this route of administration in mice [42]. Intravenous administration can be started at postnatal day 3 for rodents, which can be observed in the analyzed articles. Although, intravenous administration is the most efficient way to delivering substances, since skips the absorption phase, this route requires considerable practice given that vessels are quite small in diameter [83]. Only one of the selected studies administrated EPO locally through the left lateral ventricle. However, the authors state that local administration may not have the full benefit for animal survival compared to a systemic administration [21].

3.1.5. Disease animal model

Through the selected studies analysis, it was possible to verify that the most commonly used disease models were sepsis (n = 5), traumatic brain injury (n = 4), and autoimmune neuritis (n = 3), however, it has already been tested with significant anti-inflammatory results in several animal models including different metabolic systems, such as cardiovascular system (e.g. myocardial injury, endotoxic stroke, ischemia-reperfusion injury), nervous system (spinal cord compressive injury, intracerebral hemorrhage injury, autoimmune neuritis and encephalomyelitis, amyotrophic lateral sclerosis, peripheral inflammatory pain), reproductive system (uterus inflammation, testicular torsion), and gastrointestinal system (acute esophageal burn, acute colitis).

Sepsis is described as complex pathophysiology with systemic biochemical alterations [21,31,48]. Among them, a systemic inflammatory response is mediated by the host's immune system with a high probability of aggravating organ dysfunction and eventually, leading to death [21,31,48]. This systemic inflammatory response can be triggered by a variety of non-infectious conditions such as trauma, burns, and hemorrhagic [84]. Rapid production of cytokines, chemokines, prostaglandins, and nitric oxide are produced during sepsis [22,48]. Aoshiba et al. (2009) and Brines and Cerami [85] have already been used EPO in sepsis-induced models obtaining significant anti-inflammatory and antioxidant properties [26,27,48,85]. Furthermore, there is increasing evidence with animal studies that EPO is beneficial in sepsis since EPO attenuates the inducible nitric oxide synthase mRNA expression and the nitric oxide overproduction [26]. EPO also increases the survival rate in mice and improves endothelial function [26]. The use of the sepsis-induced model can be based on the emergency of finding alternative drugs for sepsis treatment since it is considered one of the most frequent causes of morbidity and mortality in intensive care units and a major public health concern.

The anti-inflammatory effects of EPO have also been studied in several central nervous system diseases [57–59]. In the traumatic brain injury model, activation of the innate inflammatory response is triggered, resulting in an excessive generation of reactive oxygen species, neuronal death, and membrane disruption [28,35,36]. After the brain injury, there is an increase in both pro-inflammatory and

anti-inflammatory cytokines, including infiltration of immune cells [28,34]. Yatsiv et al. (2005) and Okutan et al. (2008) proved its anti-inflammatory properties since EPO reduced the infiltration and activation of immune/inflammatory cells, like neutrophils and microglial cells [34–36,86]. The reason for using this model may be due to the fast-increased concentration of inflammatory cytokines, after the induction of the brain injury in rodents.

Experimental autoimmune neuritis is an autoantigen-specific model mediated by reactive T cells that mimic demyelinating inflammatory disease of the peripheral nervous system in humans [39]. Experimental autoimmune neuritis is characterized by breakdown of the blood-nerve barrier, accumulation of reactive T cells, macrophages, and demyelination [39]. EPO could ameliorate this autoimmune neuritis attenuating inflammation and enhancing Schwann cell as reported by Ahn et al. and Zhang et al. [39,87,88]. Experimental autoimmune neuritis is the prime animal model for inflammatory demyelinating polyneuropathies and mimics many clinical and immunological features of the human acute inflammatory demyelinating which offers the possibility to study preclinical effects of novel therapies [46,52].

3.2. Animals-related parameters

3.2.1. Animal

In rodents, mice and rats are the most used animals, but clearly, rats (n = 21) were used more often than mice (n = 16). The use of mice and rats is supported by the fact that they have many similarities with humans, namely gene homology (95%), anatomy, physiology, similar pathological response, and immune function [89–91]. Yet, as mentioned in the bibliography, mice and rats are cost-effective, which allows the use of adequate numbers for statistical relevance [90]. Animals are often preferred for study human diseases [83,90–94] and rodents present the advantage of the ease of maintenance, small size, easy handling, short life cycle, gestation time (19–21 days), and ease of breeding within the laboratory [83,90–94].

The higher use of rats may be related to the type of procedures used and the size of the animal, which can be explained by the advantage in the ability to acquire large samples for analysis and facility for surgical procedure. Though, the type of rodent does not influence the results. Studies have been done on both animals and demonstrated anti-inflammatory effects.

3.2.2. Strain

The mice used were C57BL/6 (n = 7), BALB/c (n = 3), CBA/J (n = 1), swiss albino (n = 1), sabra (n = 1), kunming (n = 1), CD-1 (n = 1) and transgenic mice (n = 1). The rats used were sprague-dawley (n = 10), wistar (n = 5), lewis (n = 4) and fisher F344 (n = 1). As evidenced, the most used strains of mice were C57BL/6, and the more common strains used of rats were sprague-dawley.

Each strain has advantages and disadvantages that often depend on the process to be studied and the choice of rodent model depends on the strains that closely reproduce the symptoms and disease process seen in humans. The reason for the use of the C57BL/6 mice strain may be explained by it is commonly used in many research areas since colonies are genetically identical within each strain. Furthermore, C57BL/6 is refractory to many tumors which can be an advantage [95,96]. The sprague-dawley strain is the most widely used rat species for biomedical research with docile nature [97]. Besides, that confers versatility, consistency, and is ideal for surgical modifications [97].

3.2.3. Gender

In these selected studies, more males (n = 21) than females (n = 6) were used for the animal disease models. Even though, some studies do not mention which gender was used (n = 9). The fact that the female gender has been less used can be justified by its production of a high number of hormones, during the estrous cycle, which can affect the experimental results [93]. Moreover, females need more time than males to recover after the anesthetic agent's application [93]. However, the use of females in some studies is explained by their use on the uterus inflammation model, when newborns are needed or when hormonal factors do not interfere with experimental results [98,99]. On the other hand, the male gender is more likely to fight each other, probably related to the establishment of hierarchy and defense of territory. That can promote cutaneous lesions with inflammatory processes involved, which can bias the anti-inflammatory findings in these particular models and, additionally, can result in an increased mortality rate not consequential from the model studied [83].

3.2.4. Age

According to the results, the age of mice usually used was between 5 and 16 weeks, corresponding to 35–112 days, and rats used were aged among 3–16 weeks, 21–112 days. Newborns mice were also used in one study with 10 days of age and three studies on rats aged between 3 and 7 days.

From day 15, mice start feeding themselves and the weaning takes place at 4 weeks of age [93]. For this reason, studies that do not need newborns should use mice from this age, which is in accordance with the results. Rats at day 16 start feeding themselves and the weaning occurs at 5 weeks of age [93]. Later compared to mice [93]. Comparing with the results, only one study used rats at 3 weeks while in the other studies rats were used from 6 weeks, as expected. One study revealed that age-related factors could interfere with the EPO response since the inflammatory effects were more pronounced in young mice (12 weeks) compared with aged mice (26 weeks) [100]. Age may have an impact on the inflammatory response since Lewis et al. [101] and Gomez et al. [100] revealed that aged mice appear to exhibit a more robust and expressive inflammatory response compared with the young mice [99–101]. In a study with adult rodents, [37] also prove that EPO partially decreases the inflammatory response in the aged rats compared with the young mice [37]. The age chosen should be according to the study but we believe that it is important to consider that aged mice may have decreased liver enzymes which can lead to toxicity.

For articles where age was not specified, the body weight was considered since provides approximately the stage of development. Based on the results obtained, male mice can reach up to 40 g and females around 30–35 g. Male rats are used between 500 and 600 g of weight and females with around 300–400 g of weight. As rodents have rapid growth, it is important to be aware of the bodyweight prior to drug administration because of the calculated drug dose.

3.3. Biomarkers assessed

The measurement of biomarkers allows a precise quantification in order to evaluate the inflammatory reaction and verify if there is a decrease in this response, after EPO administration [79]. Through the analysis of the results, the main inflammatory biomarkers measured were TNF-α (n = 17), IL-1β (n = 15), IL-6 (n = 13), IL-10 (n = 8), macrophages (n = 7), lymphocytes (n = 6), TGF-β (n = 5), microglia cells (n = 5), nitric oxide (n = 5) and IFN-ɣ (n = 5) levels.

Biomarkers indicate normal versus pathogenic conditions and assess responses for possible therapeutic interventions [102]. In the context of animal models of inflammation, the biomarkers commonly used are divide into classes such as immune-related effectors, cytokines and chemokines, reactive oxygen and nitrogen species, prostaglandins and cyclooxygenases, acute phase proteins and mediators as growth, and transcription factors [84,103,104]. These can also help to discover unknown mechanisms or to understand the pathways or molecules involved in inflammation through processes activated during inflammatory response [105].

The anti-inflammatory properties of EPO such as inhibition of leukocyte infiltration and decreased production of proinflammatory cytokines have been observed in several in vivo studies [44,106]. EPO administration has demonstrated a decrease or inhibition of

pro-inflammatory cytokines, such as TNF-α, IL-1β, IFN-ɣ, and IL-6 and an increase of anti-inflammatory cytokines, such as IL-10 and TGF-β [48].

4. Conclusions

In non-clinical studies in vivo, EPO has proved its anti-inflammatory effect through several animal models of inflammation, essentially by the monitoring of TNF-α and IL-1β biomarkers (primary inflammatory cytokines). The choice is usually the epoetin-β due to its longer half-life, so needs fewer administrations. However, if it was a long-term treatment, ARA 290 and cEPO may be a good solution since they present non-erythropoietic effects. The most used EPO dose is between 3000 and 5000 IU/kg since anti-inflammatory effects are verified with this average dose. The frequency and duration of treatment would be dependent on the type of study to be performed (acute or chronic) but a single dose would be preferable for acute models. In chronic models, the choice would be ARA 290 or cEPO. The intraperitoneal administration of EPO results in faster absorption and can be used to administer large volumes of fluid safely. EPO has been much tested in sepsis-induced models, which represent a multi-organ inflammation perfect to understand the influence of EPO in each pathophysiological process. Male rodents have been used more often, with around 5 weeks (C57BL/6 mice) or 6 weeks (Sprague-Dawley rats).

EPO has been recognized as a multifunctional cytokine with anti-inflammatory properties and for this reason, this molecule is an interesting candidate to treat inflammatory-based diseases, improving patient's quality of life. Hence, as future perspectives the enlightenment of all mechanisms of EPO in inflammation such as the role of JAK2/STAT3/5 and NF-κB pathways require further studies to allow the translation of this molecule to the clinical practice as an approved treatment for inflammation.

Funding

This work was supported by Polytechnic Institute of Lisbon [grant numbers IPL/2020/cEPO_ESTeSL].

Author contributions

IS and CA carried out the systematic review and wrote the manuscript with support from RP and VM. VM conceived the original idea and supervised the project.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgments

H&TRC authors gratefully acknowledge the FCT/MCTES national support through the UIDB/05608/2020 and UIDP/05608/2020.

References

[1] W. Jelkmann, Regulation of erythropoietin production, J. Physiol. 589 (2011) 1251–1258, https://doi.org/10.1113/jphysiol.2010.195057.
[2] B. Peng, G. Kong, C. Yang, Y. Ming, Erythropoietin and its derivatives: from tissue protection to immune regulation, Cell Death Dis. 11 (2020) 2–12, https://doi.org/10.1038/s41419-020-2276-8.
[3] M.O. Arcasoy, Non-erythroid effects of erythropoietin, Haematologica 95 (2010) 1803–1805, https://doi.org/10.3324/haematol.2010.030213.
[4] G.K. Thilaka, S.V. Kumar, A review on pharmacological use of recombinant human erythropoietin in renal and nonrenal anemia and other potential applications in clinical practice, Apollo Med. 13 (2016) 80–85, https://doi.org/10.1016/j.apme.2016.01.004.
[5] R.M. López, B.S. Aladrén, F.G. García, Use of agents stimulating erythropoiesis in digestive diseases, World J. Gastroenterol. 15 (2009) 4675–4685, https://doi.org/10.3748/wjg.15.4675.
[6] E. Obeagu, A review on erythropoietin, Int. J. Adv. Res. Biol. Sci. 2 (2015) 35–47.
[7] C.G. Winearls, Erythropoietin, Nephrol. Dial. Transplant. 4 (1989) 323–326, https://doi.org/10.1093/oxfordjournals.ndt.a091884.
[8] S. Suresh, P.K. Rajvanshi, C.T. Noguchi, The many facets of erythropoietin physiologic and metabolic response, Front. Physiol. 10 (2020) 1–20, https://doi.org/10.3389/fphys.2019.01534.
[9] J. Vogel, M. Gassmann, Erythropoietic and non-erythropoietic functions of erythropoietin in mouse models, J. Physiol. 589 (2011) 1259–1264, https://doi.org/10.1113/jphysiol.2010.196147.
[10] S. Elliott, E. Pham, I.C. Macdougall, Erythropoietins: a common mechanism of action, Exp. Hematol. 36 (2008) 1573–1584, https://doi.org/10.1016/j.exphem.2008.08.003.
[11] M.C. Rivera-Cervantes, J.J. Jarero-Basulto, J. Murguía-Castillo, A.G. Marín-López, Y. Gasca-Martínez, S. Cornelio-Martínez, C. Beas-Zárate, The recombinant human erythropoietin administered in neonatal rats after excitotoxic damage induces molecular changes in the hippocampus, Front. Neurosci. 13 (2019) 1–13, https://doi.org/10.3389/fnins.2019.00118.
[12] S. Chateauvieux, C. Grigorakaki, F. Morceau, M. Dicato, M. Diederich, Erythropoietin, erythropoiesis and beyond, Biochem. Pharmacol. 82 (2011) 1291–1303, https://doi.org/10.1016/j.bcp.2011.06.045.
[13] W. Jelkmann, Molecular biology of erythropoietin, Intern. Med. 43 (2004) 649–659, https://doi.org/10.2169/internalmedicine.43.649.
[14] K.R. Patil, U.B. Mahajan, B.S. Unger, S.N. Goyal, S. Belemkar, S.J. Surana, S. Ojha, C.R. Patil, Animal models of inflammation for screening of anti-inflammatory drugs: implications for the discovery and development of phytopharmaceuticals, Int. J. Mol. Sci. 20 (2019) 4367, https://doi.org/10.3390/ijms20184367.
[15] A.F. Mendes, M.T. Cruz, O. Gualillo, The physiology of inflammation—the final common pathway to disease, Front. Physiol. 9 (2018) 1–3, https://doi.org/10.3389/fphys.2018.01741.
[16] R.H. Straub, C. Schradin, Chronic inflammatory systemic diseases – an evolutionary trade-off between acutely beneficial but chronically harmful programs, Evol. Med. Public Heal. 2016 (2016) 37–51, https://doi.org/10.1093/emph/eow001.
[17] C.A. Dinarello, Anti-inflammatory agents: present and future, Cell 140 (2010) 935–950, https://doi.org/10.1016/j.cell.2010.02.043.
[18] L.A. Abdulkhaleq, M.A. Assi, R. Abdullah, M. Zamri-Saad, Y.H. Taufiq-Yap, M.N. M. Hezmee, The crucial roles of inflammatory mediators in inflammation: a review, Vet. World 11 (2018) 627–635, https://doi.org/10.14202/vetworld.2018.627-635.
[19] M.W. Konstan, P.B. Davis, Pharmacological approaches for the discovery and development of new anti-inflammatory agents for the treatment of cystic fibrosis, Adv. Drug Deliv. Rev. 54 (2002) 1409–1423, https://doi.org/10.1016/S0169-409X(02)00146-1.
[20] V. Mateus, J. Rocha, P. Alves, H. Mota-Filipe, B. Sepodes, R.M.A. Pinto, Anti-inflammatory effect of erythropoietin in the TNBS-induced colitis, Basic Clin. Pharmacol. Toxicol. 120 (2017) 138–145, https://doi.org/10.1111/bcpt.12663.
[21] T.-F. Zhou, J.-G. Yu, Recombinant human erythropoietin attenuates neuronal apoptosis and cognitive defects via JAK2/STAT3 signaling in experimental endotoxemia, J. Surg. Res. 183 (2013) 304–312, https://doi.org/10.1016/j.jss.2012.11.035.
[22] C.M. Comim, O.J. Cassol, I. Abreu, T. Moraz, L.S. Constantino, F. Vuolo, L. S. Galant, N. de Rochi, M.O. dos Santos Morais, G. Scaini, T. Barichello, E. L. Streck, J. Quevedo, F. Dal-Pizzol, Erythropoietin reverts cognitive impairment and alters the oxidative parameters and energetic metabolism in sepsis animal model, J. Neural Transm. 119 (2012) 1267–1274, https://doi.org/10.1007/s00702-012-0774-2.
[23] X. Zhang, S. Dong, Y. Qin, X. Bian, Protective effect of erythropoietin against myocardial injury in rats with sepsis and its underlying mechanisms, Mol. Med. Rep. 11 (2015) 3317–3329, https://doi.org/10.3892/mmr.2015.3155.
[24] C. Tsompos, C. Panoulis, K. Toutouzas, A. Triantafyllou, G. Zografos, A. Papalois, The effect of erythropoietin on uterus inflammation during ischemia reperfusion injury in rats, Ceska Gynekol. 81 (2016) 342–348.
[25] V. Bakan, M. Garipardıc, M. Okumuş, H. Çıralik, Y. Atlı, D. Özbağ, F.I. Tolun, The protective effect of erythropoietin on the acute phase of corrosive esophageal burns in a rat model, Pediatr. Surg. Int. 26 (2010) 195–201, https://doi.org/10.1007/s00383-009-2480-1.
[26] K. Kandasamy, S. Choudhury, V. Singh, M.P. Addison, S.A. Darzi, J.K. Kasa, R. Thangamalai, J.R. Dash, T. Kumar, F. Sultan, T.U. Singh, S. Parida, S.K. Mishra, Erythropoietin reverses sepsis-induced vasoplegia to norepinephrine through preservation of α1D-adrenoceptor mRNA expression and inhibition of GRK2-mediated desensitization in mouse aorta, J. Cardiovasc. Pharmacol. Ther. 21 (2016) 100–113, https://doi.org/10.1177/1074248415587968.
[27] K. Aoshiba, S. Onizawa, T. Tsuji, A. Nagai, Therapeutic effects of erythropoietin in murine models of endotoxin shock, Crit. Care Med. 37 (2009) 889–898, https://doi.org/10.1097/CCM.0b013e31819b8371.
[28] G.D. Anderson, T.C. Peterson, C. Vonder Haar, F.M. Farin, T.K. Bammler, J. W. MacDonald, E.D. Kantor, M.R. Hoane, Effect of traumatic brain injury, erythropoietin, and anakinra on hepatic metabolizing enzymes and transporters in an experimental rat model, AAPS J. 17 (2015) 1255–1267, https://doi.org/10.1208/s12248-015-9792-y.
[29] P. Thériault, A. Le Béhot, A. ElAli, S. Rivest, Sub-acute systemic erythropoietin administration reduces ischemic brain injury in an age-dependent manner, Oncotarget 7 (2016) 35552–35561, https://doi.org/10.18632/oncotarget.9652.
[30] M. Kapan, A. Onder, H. Yuksel, O. Evliyaoglu, U. Firat, R. Tekin, M. Gul, I. Aliosmanoglu, The effects of erythropoietin on bacterial translocation and

inflammation in rats with obstructive jaundice, J. Med. Biochem. 32 (2013) 39–46, https://doi.org/10.2478/v10011-012-0001-6.
[31] X. Shi, F. Yang, Y.N. Zheng, H. Zhang, X.X. Wang, G.J. Shao, X.L. Lai, Metabolomic approach for the identification of therapeutic targets of erythropoietin against sepsis in rat models, Eur. Rev. Med. Pharmacol. Sci. 20 (2016) 537–546.
[32] C. Savino, R. Pedotti, F. Baggi, F. Ubiali, B. Gallo, S. Nava, P. Bigini, S. Barbera, E. Fumagalli, T. Mennini, A. Vezzani, M. Rizzi, T. Coleman, A. Cerami, M. Brines, P. Ghezzi, R. Bianchi, Delayed administration of erythropoietin and its non-erythropoietic derivatives ameliorates chronic murine autoimmune encephalomyelitis, J. Neuroimmunol. 172 (2006) 27–37, https://doi.org/10.1016/j.jneuroim.2005.10.016.
[33] L.J. Ramírez-Jirano, T. Zenteno-Savín, R. Gaxiola-Robles, E.J. Ramos-González, B.M. Torres-Mendoza, O.K. Bitzer-Quintero, The neuroprotective effect of erythropoietin in rat hippocampus in an endotoxic shock model, Rev. Investig. Clin. 68 (2016) 292–298.
[34] Z. Zhou, F. Li, Z. Zheng, Y. Li, T. Chen, W. Gao, J. Chen, J. Zhang, Erythropoietin regulates immune/inflammatory reaction and improves neurological function outcomes in traumatic brain injury, Brain Behav. 7 (2017) 1–10, https://doi.org/10.1002/brb3.827.
[35] I. Yatsiv, N. Grigoriadis, C. Simeonidou, P.F. Stahel, O.I. Schmidt, A. G. Alexandrovich, J. Tsenter, E. Shohami, Erythropoietin is neuroprotective, improves functional recovery, and reduces neuronal apoptosis and inflammation in a rodent model of experimental closed head injury, FASEB J. 19 (2005) 1–20, https://doi.org/10.1096/fj.05-3907fje.
[36] S.E. Juul, R.P. Beyer, T.K. Bammler, R.J. McPherson, J. Wilkerson, F.M. Farin, Microarray analysis of high-dose recombinant erythropoietin treatment of unilateral brain injury in neonatal mouse hippocampus, Pediatr. Res. 65 (2009) 485–492, https://doi.org/10.1203/PDR.0b013e31819d90c8.
[37] M. Chau, D. Chen, L. Wei, Erythropoietin attenuates inflammatory factors and cell death in neonatal rats with intracerebral hemorrhage, Intracerebral Hemorrhage Res. 111 (2011) 299–305, https://doi.org/10.1007/978-3-7091-0693-8_50.
[38] D. Mihov, N. Bogdanov, B. Grenacher, M. Gassmann, G. Zünd, A. Bogdanova, R. Tavakoli, Erythropoietin protects from reperfusion-induced myocardial injury by enhancing coronary endothelial nitric oxide production, Eur. J. Cardio Thorac. Surg. 35 (2009) 839–846, https://doi.org/10.1016/j.ejcts.2008.12.049.
[39] B. Luo, M. Jiang, X. Yang, Z. Zhang, J. Xiong, H.J. Schluesener, Z. Zhang, Y. Wu, Erythropoietin is a hypoxia inducible factor-induced protective molecule in experimental autoimmune neuritis, Biochim. Biophys. 1832 (2013) 1260–1270, https://doi.org/10.1016/j.bbadis.2013.04.015.
[40] O. Mohamad, D. Chen, L. Zhang, C. Hofmann, L. Wei, S.P. Yu, Erythropoietin reduces neuronal cell death and hyperalgesia induced by peripheral inflammatory pain in neonatal rats, Mol. Pain. 7 (2011) 1–15, https://doi.org/10.1186/1744-8069-7-51.
[41] S.-H. Koh, Y. Kim, H.Y. Kim, G. Won Cho, K.S. Kim, S.H. Kim, Recombinant human erythropoietin suppresses symptom onset and progression of G93A-SOD1 mouse model of ALS by preventing motor neuron death and inflammation, Eur. J. Neurosci. 25 (2007) 1923–1930, https://doi.org/10.1111/j.1460-9568.2007.05471.x.
[42] R. Yuan, Y. Maeda, W. Li, W. Lu, S. Cook, P. Dowling, Erythropoietin: a potent inducer of peripheral immuno/inflammatory modulation in autoimmune EAE, PLoS One 3 (2008) 1–11, https://doi.org/10.1371/journal.pone.0001924.
[43] M. Heitrich, D.M. de los Á. García, T.R. Stoyanoff, J.P. Rodríguez, J.S. Todaro, M. V. Aguirre, Erythropoietin attenuates renal and pulmonary injury in polymicrobial induced-sepsis through EPO-R, VEGF and VEGF-R2 modulation, Biomed. Pharm. 82 (2016) 606–613, https://doi.org/10.1016/j.biopha.2016.05.045.
[44] J.H. Lee, D.K. Sung, S.H. Koo, B.K. Shin, Y.S. Hong, C.S. Son, J.W. Lee, Y. S. Chang, W.S. Park, Erythropoietin attenuates hyperoxia-induced lung injury by down-modulating inflammation in neonatal rats, J. Korean Med. Sci. 22 (2007) 1042–1047, https://doi.org/10.3346/jkms.2007.22.6.1042.
[45] N. Yazihan, H. Ataoglu, N. Koku, E. Erdemli, A.K. Sargin, Protective role of erythropoietin during testicular torsion of the rats, World J. Urol. 25 (2007) 531–536, https://doi.org/10.1007/s00345-007-0200-9.
[46] A.K. Mausberg, G. Meyer zu Hörste, T. Dehmel, M. Stettner, H.C. Lehmann, K. A. Sheikh, B.C. Kieseier, Erythropoietin ameliorates rat experimental autoimmune neuritis by inducing transforming growth factor-beta in macrophages, PLoS One 6 (2011) 1–8, https://doi.org/10.1371/journal.pone.0026280.
[47] C. Hempel, V. Combes, N.H. Hunt, J.A.L. Kurtzhals, G.E.R. Grau, CNS hypoxia is more pronounced in murine cerebral than noncerebral malaria and is reversed by erythropoietin, Am. J. Pathol. 179 (2011) 1939–1950, https://doi.org/10.1016/j.ajpath.2011.06.027.
[48] I. Ates, N. Dogan, M. Aksoy, Z. Halıcı, C. Gündogdu, M.S. Keles, The protective effects of IgM-enriched immunoglobulin and erythropoietin on the lung and small intestine tissues of rats with induced sepsis: Biochemical and histopathological evaluation, Pharm. Biol. 53 (2015) 78–84, https://doi.org/10.3109/13880209.2014.910535.
[49] Z. Eren, J. Çoban, I.D. Ekinci, C. Kaspar, G. Kantarci, Evaluation of the effects of a high dose of erythropoietin-beta on early endotoxemia using a rat model, Adv. Clin. Exp. Med. 21 (2012) 321–329.
[50] K. Kaiser, A. Texier, J. Ferrandiz, A. Buguet, A. Meiller, C. Latour, F. Peyron, R. Cespuglio, S. Picot, Recombinant human erythropoietin prevents the death of mice during cerebral malaria, J. Infect. Dis. 193 (2006) 987–995, https://doi.org/10.1086/500844.
[51] M. Sigal, N. Siebert, D. Zechner, E. Menschikow, K. Abshagen, B. Vollmar, C. Eipel, Darbepoetin-α inhibits the perpetuation of necro-inflammation and delays the progression of cholestatic fibrosis in mice, Lab. Investig. 90 (2010) 1447–1456, https://doi.org/10.1038/labinvest.2010.115.
[52] Y. Liu, B. Luo, F. Han, X. Li, J. Xiong, M. Jiang, X. Yang, Y. Wu, Z. Zhang, Erythropoietin-derived nonerythropoietic peptide ameliorates experimental autoimmune neuritis by inflammation suppression and tissue protection, PLoS One 9 (2014) 1–11, https://doi.org/10.1371/journal.pone.0090942.
[53] B. Huang, J. Jiang, B. Luo, W. Zhu, Y. Liu, Z. Wang, Z. Zhang, Non-erythropoietic erythropoietin-derived peptide protects mice from systemic lupus erythematosus, J. Cell. Mol. Med. 22 (2018) 3330–3339, https://doi.org/10.1111/jcmm.13608.
[54] W.N. Nijboer, P.J. Ottens, A. van Dijk, H. van Goor, R.J. Ploeg, H.G.D. Leuvenink, Donor pretreatment with carbamylated erythropoietin in a brain death model reduces inflammation more effectively than erythropoietin while preserving renal function, Crit. Care Med. 38 (2010) 1155–1161, https://doi.org/10.1097/CCM.0b013e3181cf6e78.
[55] W. Liu, Y. Shen, J.M. Plane, D.E. Pleasure, W. Deng, Neuroprotective potential of erythropoietin and its derivative carbamylated erythropoietin in periventricular leukomalacia, Exp. Neurol. 230 (2011) 227–239, https://doi.org/10.1016/j.expneurol.2011.04.021.
[56] W. Jelkmann, Physiology and pharmacology of erythropoietin, Transfus. Med. Hemother. 40 (2013) 302–309, https://doi.org/10.1159/000356193.
[57] V. Skibeli, G. Nissen-Lie, P. Torjesen, Sugar profiling proves that human serum erythropoietin differs from recombinant human erythropoietin, Blood 98 (2001) 3626–3634, https://doi.org/10.1182/blood.V98.13.3626.
[58] J. Azmandian, M.R. Abbasi, V. Pourfarziani, A.A. Nasiri, S. Ossareh, S. Ezzatzadegan Jahromi, H. Sanadgol, S. Amini, A.S. Farahani, Comparing therapeutic efficacy and safety of epoetin beta and epoetin alfa in the treatment of anemia in end-stage renal disease hemodialysis patients, Am. J. Nephrol. 48 (2018) 251–259, https://doi.org/10.1159/000493097.
[59] B. Cowper, M. Lavén, B. Hakkarainen, E. Mulugeta, Glycan analysis of erythropoiesis-stimulating agents, J. Pharm. Biomed. Anal. 180 (2020) 1–7, https://doi.org/10.1016/j.jpba.2019.113031.
[60] F.E. Tögel, J.D. Ahlstrom, Y. Yang, Z. Hu, P. Zhang, C. Westenfelder, Carbamylated erythropoietin outperforms erythropoietin in the treatment of AKI-on-CKD and other AKI models, J. Am. Soc. Nephrol. 27 (2016) 3394–3404, https://doi.org/10.1681/ASN.2015091059.
[61] N.K. Tiwari, M. Sathyanesan, W. Schweinle, S.S. Newton, Carbamoylated erythropoietin induces a neurotrophic gene profile in neuronal cells, Prog. Neuro Psychopharmacol. Biol. Psychiatry 88 (2019) 132–141, https://doi.org/10.1016/j.pnpbp.2018.07.011.
[62] J. Chen, Z. Yang, X. Zhang, Carbamylated erythropoietin: a prospective drug candidate for neuroprotection, Biochem. Insights 8 (2015) 25–29, https://doi.org/10.4137/BCI.S40221.
[63] M. Diao, Y. Qu, H. Liu, Y. Ma, X. Lin, Effect of carbamylated erythropoietin on neuronal apoptosis in fetal rats during intrauterine hypoxic-ischemic encephalopathy, Biol. Res. 52 (2019) 28, https://doi.org/10.1186/s40659-019-0234-7.
[64] R.E. Maltaneri, M.E. Chamorro, A. Schiappacasse, A.B. Nesse, D.C. Vittori, Differential effect of erythropoietin and carbamylated erythropoietin on endothelial cell migration, Int. J. Biochem. Cell Biol. 85 (2017) 25–34, https://doi.org/10.1016/j.biocel.2017.01.013.
[65] D. Ostrowski, R. Heinrich, Alternative erythropoietin receptors in the nervous system, J. Clin. Med. 7 (2018) 24, https://doi.org/10.3390/jcm7020024.
[66] M. Leist, Derivatives of erythropoietin that are tissue protective but not erythropoietic, Science 80- (305) (2004) 239–242, https://doi.org/10.1126/science.1098313.
[67] M. Niesters, M. Swartjes, L. Heij, M. Brines, A. Cerami, A. Dunne, E. Hoitsma, A. Dahan, The erythropoietin analog ARA 290 for treatment of sarcoidosis-induced chronic neuropathic pain, Expert Opin. Orphan Drugs 1 (2013) 77–87, https://doi.org/10.1517/21678707.2013.719289.
[68] M. Brines, A.N. Dunne, M. van Velzen, P.L. Proto, C. Ostenson, R.I. Kirk, I. N. Petropoulos, S. Javed, R.A. Malik, A. Cerami, A. Dahan, ARA 290, a nonerythropoietic peptide engineered from erythropoietin, improves metabolic control and neuropathic symptoms in patients with type 2 diabetes, Mol. Med. 20 (2014) 658–666, https://doi.org/10.2119/molmed.2014.00215.
[69] M. Swartjes, M. van Velzen, M. Niesters, L. Aarts, M. Brines, A. Dunne, A. Cerami, A. Dahan, ARA 290, a peptide derived from the tertiary structure of erythropoietin, produces long-term relief of neuropathic pain coupled with suppression of the spinal microglia response, Mol. Pain. 10 (2014) 1–12, https://doi.org/10.1186/1744-8069-10-13.
[70] G. Cerri, M. Montagna, L. Madaschi, D. Merli, P. Borroni, F. Baldissera, A. Gorio, Erythropoietin effect on sensorimotor recovery after contusive spinal cord injury: an electrophysiological study in rats, Neuroscience 219 (2012) 290–301, https://doi.org/10.1016/j.neuroscience.2012.05.041.
[71] A. Çetin, K. Nas, H. Büyükbayram, A. Ceviz, G. Ölmez, The effects of systemically administered methylprednisolone and recombinant human erythropoietin after acute spinal cord compressive injury in rats, Eur. Spine J. 15 (2006) 1539–1544, https://doi.org/10.1007/s00586-006-0091-2.
[72] W. Jin, X. Ming, X. Hou, T. Zhu, B. Yuan, J. Wang, H. Ni, J. Jiang, H. Wang, W. Liang, Protective effects of erythropoietin in traumatic spinal cord injury by inducing the Nrf2 signaling pathway activation, J. Trauma Acute Care Surg. 76 (2014) 1228–1234, https://doi.org/10.1097/TA.0000000000000211.
[73] E. Kaptanoglu, I. Solaroglu, O. Okutan, H.S. Surucu, F. Akbiyik, E. Beskonakli, Erythropoietin exerts neuroprotection after acute spinal cord injury in rats: effect

on lipid peroxidation and early ultrastructural findings, Neurosurg. Rev. 27 (2004) 113–120, https://doi.org/10.1007/s10143-003-0300-y.
[74] A.C.C.P. de Souza, R.A. Volpini, M.H. Shimizu, T.R. Sanches, N.O.S. Camara, P. Semedo, C.E. Rodrigues, A.C. Seguro, L. Andrade, Erythropoietin prevents sepsis-related acute kidney injury in rats by inhibiting NF-κB and upregulating endothelial nitric oxide synthase, Am, J. Physiol. Physiol. 302 (2012) F1045–F1054, https://doi.org/10.1152/ajprenal.00148.2011.
[75] S. Mori, T. Sawada, T. Okada, K. Kubota, Erythropoietin and its derivative protect the intestine from severe ischemia/reperfusion injury in the rat, Surgery 143 (2008) 556–565, https://doi.org/10.1016/j.surg.2007.12.013.
[76] Z. Jia, R. Xue, S. Ma, J. Xu, S. Guo, S. Li, E. Zhang, J. Wang, J. Yang, Erythropoietin attenuates the memory deficits in aging rats by rescuing the oxidative stress and inflammation and promoting BDNF releasing, Mol. Neurobiol. 53 (2016) 5664–5670, https://doi.org/10.1007/s12035-015-9438-1.
[77] C.J. Parsa, A. Matsumoto, J. Kim, R.U. Riel, L.S. Pascal, G.B. Walton, R. B. Thompson, J.A. Petrofski, B.H. Annex, J.S. Stamler, W.J. Koch, A novel protective effect of erythropoietin in the infarcted heart, J. Clin. Invest. 112 (2003) 999–1007, https://doi.org/10.1172/JCI18200.
[78] M. Patel, G. Murunganathan, K.P.S. Gowda, In vivo animal models in preclinical evaluation of anti- inflammatory activity-a review, Int. J. Pharm. Res. Allied Sci. 1 (2012) 1–5. ⟨www.ijpras.com⟩.
[79] Y.-K. Chang, D.E. Choi, K.-R. Na, S.-J. Lee, K.-S. Suh, S.Y. Kim, Y.-T. Shin, K. W. Lee, Erythropoietin attenuates renal injury in an experimental model of rat unilateral ureteral obstruction via anti-inflammatory and anti-apoptotic effects, J. Urol. 181 (2009) 1434–1443, https://doi.org/10.1016/j.juro.2008.10.105.
[80] T.R. Coleman, C. Westenfelder, F.E. Tögel, Cytoprotective doses of erythropoietin or carbamylated erythropoietin have markedly different procoagulant and vasoactive activities, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 5965–5970.
[81] Y. Xiong, A. Mahmood, Y. Zhang, Effects of posttraumatic carbamylated erythropoietin therapy on reducing lesion volume and hippocampal cell loss, enhancing angiogenesis and neurogenesis, and improving functional outcome in rats following traumatic brain injury, J. Neurosurg. 114 (2011) 549–559, https://doi.org/10.3171/2010.10.JNS10925.
[82] J. Ding, J. Wang, Q.Y. Li, Neuroprotection and CD131/GDNF/AKT pathway of carbamylated erythropoietin in hypoxic neurons, Mol. Neurobiol. 54 (2017) 5051–5060, https://doi.org/10.1007/s12035-016-0022-0.
[83] M.A. Suckow, P. Danneman, C. Brayton, The Laboratory Mouse, CRC Press, Boca Raton, 2001.
[84] W. Schulte, J. Bernhagen, R. Bucala, Cytokines in sepsis: potent immunoregulators and potential therapeutic targets—an updated view, Mediat. Inflamm. 2013 (2013) 1–16, https://doi.org/10.1155/2013/165974.
[85] M. Brines, A. Cerami, Discovering erythropoietin's extra-hematopoietic functions: biology and clinical promise, Kidney Int. 70 (2006) 246–250, https://doi.org/10.1038/sj.ki.5001546.
[86] O. Okutan, O.F. Turkoglu, H.B. Gok, E. Beskonakli, Neuroprotective effect of erythropoietin after experimental cold injury–induced vasogenic brain edema in rats, Surg. Neurol. 70 (2008) 498–502, https://doi.org/10.1016/j.surneu.2007.07.061.
[87] Z.-S. Yin, H. Zhang, W. Gao, Erythropoietin promotes functional recovery and enhances nerve regeneration after peripheral nerve injury in rats, Am. J. Neuroradiol. 31 (2010) 509–515, https://doi.org/10.3174/ajnr.A1820.
[88] M. Ahn, C. Moon, C. Jeong, Y. Matsumoto, C.-S. Koh, T. Shin, Upregulation of erythropoietin in rat peripheral nervous system with experimental autoimmune neuritis, Brain Res 1333 (2010) 82–90, https://doi.org/10.1016/j.brainres.2010.03.072.
[89] P. McGonigle, B. Ruggeri, Animal models of human disease: challenges in enabling translation, Biochem. Pharmacol. 87 (2014) 162–171, https://doi.org/10.1016/j.bcp.2013.08.006.
[90] E.C. Bryda, The mighty mouse: the impact of rodents on advances in biomedical research, Mo. Med. 110 (2013) 207–211.
[91] R.H. Waterston, K. Lindblad-Toh, E. Birney, J. Rogers, J.F. Abril, Initial sequencing and comparative analysis of the mouse genome, Nature 420 (2002) 520–562, https://doi.org/10.1038/nature01262.
[92] S.E. Park, T.P. Schaer, Preclinical animal models, Acad. Entrep. Med. Heal. Sci. 1 (2019), https://doi.org/10.1371/journal.pbio.2003693.
[93] A. Andrade, S.C. Pinto, R.S. de Oliveira, Animais de Laboratório: criação e experimentação, Ed. Fiocruz (2002), https://doi.org/10.7476/9788575413869.
[94] J.A. Jiminez, T.C. Uwiera, G. Douglas Inglis, R.R.E. Uwiera, Animal models to study acute and chronic intestinal inflammation in mammals, Gut Pathog. 7 (2015) 29, https://doi.org/10.1186/s13099-015-0076-y.
[95] T.J. Laboratory, C57BL/6J, (n.d.). https://www.jax.org/strain/000664.
[96] Charles River, C57BL/6 Mouse, (n.d.). https://www.criver.com/products-services/find-model/c57bl6-mouse?region=3616.
[97] T. Biosciences, Sprague Dawley Rat, n.d.
[98] W.W. Beatty, Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organizational and activational influences, Horm. Behav. 12 (1979) 112–163, https://doi.org/10.1016/0018-506X(79)90017-5.
[99] F. Liu, L.D. McCullough, Interactions between age, sex, and hormones in experimental ischemic stroke, Neurochem. Int. 61 (2012) 1255–1265, https://doi.org/10.1016/j.neuint.2012.10.003.Interactions.
[100] C.R. Gomez, S. Hirano, B.T. Cutro, S. Birjandi, H. Baila, V. Nomellini, E.J. Kovacs, Advanced age exacerbates the pulmonary inflammatory response after lipopolysaccharide exposure, Crit. Care Med. 35 (2007) 246–251, https://doi.org/10.1097/01.CCM.0000251639.05135.E0.
[101] D.K. Lewis, K.T. Thomas, A. Selvamani, F. Sohrabji, Age-related severity of focal ischemia in female rats is associated with impaired astrocyte function, Neurobiol. Aging 33 (2012) 1123.e1–1123.e16, https://doi.org/10.1016/j.neurobiolaging.2011.11.007.
[102] L. Chen, H. Deng, H. Cui, J. Fang, Z. Zuo, J. Deng, Y. Li, X. Wang, L. Zhao, Inflammatory responses and inflammation-associated diseases in organs, Oncotarget 9 (2018) 7204–7218, https://doi.org/10.18632/oncotarget.23208.
[103] D.R. Brenner, D. Scherer, K. Muir, J. Schildkraut, M.R. Spitz, L. Lemarchand, A. T. Chan, E.L. Goode, C.M. Ulrich, R.J. Hung, A review of the application of inflammatory biomarkers in epidemiologic cancer research, Cancer Epidemiol. Biomarkers Prev. 23 (2014) 1729–1751, https://doi.org/10.1158/1055-9965.EPI-14-0064.A.
[104] N.L. Halladin, Oxidative and inflammatory biomarkers of ischemia and reperfusion injuries, Dan. Med. J. 62 (2015) 1–22.
[105] T. Rodney, N. Osier, J. Gill, Pro- and anti-inflammatory biomarkers and traumatic brain injury outcomes: a review, Cytokine 110 (2018) 248–256, https://doi.org/10.1016/j.cyto.2018.01.012.
[106] S. Cuzzocrea, R. Di Paola, E. Mazzon, N.S.A. Patel, T. Genovese, C. Muià, C. Crisafulli, A.P. Caputi, C. Thiemermann, Erythropoietin reduces the development of nonseptic shock induced by zymosan in mice, Crit. Care Med. 34 (2006) 1168–1177, https://doi.org/10.1097/01.CCM.0000207346.56477.E8.