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Review Article

Pleithropic Effects of Erythropoietin and Its Potential use in Plastic Surgery

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Introduction

Erythropoietin (EPO) is known to be a body-own hormone which is produced depending on the oxygen partial pressure in the blood by fibroblasts of the kidney parenchyma and controls the differentiation of the erythrocytes in the bone marrow.

The need for the existence of such a hormone had been postulated more than a century ago [1]. But only a good half-century ago (1953) the glycoprotein could be isolated and its erythropoietic effect proved [2]. After more than a quarter of a century (1984), genetic modification of hamster cells succeeded in producing recombinant human EPO [3]. Thus, there was nothing in the way of a large-scale industrial, pharmaceutical production. Now, for almost three decades, EPO has become an indispensable part of the clinical routine, for example, in the case of anemias of terminal renal failure, tumor-induced anaemias and pre-blood donation [4]. In this respect, it is important to note that the erythropoietic effects are generally observed only after a certain dosis and secondly in repeated applications of systemically administered EPO [4].

The first work on the non-erythropoietic effects of EPO was published around the turn of the Millennium [5]. Since then, our understanding of EPO has been changing.

In the meantime, a number of works have already demonstrated that EPO plays a role in responding to acute and chronic tissue damage. It was shown in several rodent models that EPO is synthesized in a variety of tissues in the acute phase after trauma. There, it inhibits the initial inflammatory reaction and thereby facilitates the healing and thus the "Restitutio ad Integrity" [6].

Various EPO effects have already been described in the regenerative phases of wound healing in rodent models, following the acute inflammatory phase. These include, for example, inhibition of apoptosis [7,8], stem cell recruitment [9] and influencing the distribution of various growth factors [10], but also the stimulation of angiogenesis and re-epithelialization [10].

The first works, which dealt with the post-traumatic effects of EPO, describes the anti-inflammatory effects in the gastrointestinal tract [11], in the lungs and in the central nervous system [12-14]. It was followed very soon by studies on other organ systems and tissues, such as the myocardial [15], the liver [16,17] and the kidney parenchyma [18].

Comprehensive reviews of the acute and long-term anti-inflammatory and pro-regenerative effects of EPO are published in two reviews by Brines and Cerami as well as by Arcasoy [19,20].

The different effects of EPO within the various organ systems (erythropoiesis, versus anti-inflammatory and pro-regenerative effects) can most likely be explained through a different affinity to the individual receptor types and receptor subtypes [20]. The tissue protective properties are mediated by an EPO hetero receptor, whose affinity to EPO is less than that of the EPOR2 receptor, which mediates the erythropoietic effect of EPO. The EPO hetero receptor is usually not found in healthy tissues, but in post-traumatic settings it has been described several times [19]. This could explain, among other things, why EPO works only after trauma pro-regenerative and pro-proliferative [6]. Since the detection of the EPO hetero receptor has not been successful so far (see below) this approach is still being discussed in a controversial way.

A major problem in researching the non-hematopoietic effects of EPO is that the existing antibodies to the EPO hetero receptor are unlikely to be specific. It turned out that the antibodies used also bind to other cytokine receptors, making it difficult to interpret the results. Accordingly, at least some of the results described here are tentatively visible [21,22]. It is therefore particularly important in the future to re-evaluate the present results with new, highly specific antibodies whose development is being intensively worked on. Ultimately, it will only be clarified with the help of a specific antibody whether a separate EPO-hetero-receptor exists and in which molecular signal pathway it plays a role.

In addition to the desired effects of EPO, undesirable effects are also described [4]. Three of these are known only for longer term EPO therapy: an increased risk for thrombosis [23,24], development of antibodys [25] against RhEPO and a blood pressure increase [26,27]. However, with regard to the thrombophilia by EPO, the working group around Corwin showed that, with appropriate, weight-adapted thrombosis prophylaxis using low-molecular heparin, the thrombosis tendency under EPO therapy is no greater than in the Control group without EPO administration [28].

The problem of rising blood pressure values has so far only been described in patients who had already shown blood pressure abnormalities in advance. In this case, an acceptable risk minimization should be feasible through careful evaluation and selection of the patients.

A problem that has not yet been conclusively discussed is the treatment of tumor anemia with EPO. EPO is generally approved for the treatment of tumor anemia. In the meantime, however, a large number of works have shown that EPO therapy has a negative impact on patients lifetimes [27,29,30]. The discussions, whether this

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is due solely to the optimized oxgenization to anemia or to the pro-angiogenic and anti-apoptotic or the pro-thrombotic effects of EPO, could not be completed so far.

However, a direct, active oncogenic effect of EPO has not yet been shown [29,30].

In the field of plastic surgery, some interesting new applications for EPO have been described in recent years.

Tissue Ischemia and Flap Surgery

Based on the assumption that EPO could act as a tissue-protective in ischemic damage, the examination in experimental flaps, which are modelled, among other things, with a reproducible zone of persistent ischemia with subsequent necrosis, was published.

As early as 2003, Saray and Employees [31] published a first work on this, which dealt with the use of EPO to improve the flap survival in the case of randomly perfused lobes (McFarlane type) in the rat model. On the one side, the flap survival was investigated, as well as potential adverse effects induced by EPO, such as hematocrit and blood pressure rises. Over 3 weeks, i.e. long-term administered low-dose EPO (50mg/kg body weight) and only 1 week, i.e. short-term low and high-dose EPO, was accompanied by a statistically significantly improved flap survival [31]. A statistically significant increase in hematocrit and blood pressure could only be detected in animals that had received over three weeks of high-dose EPO (150mg/kg body weight (kg). This significant increase correlated with a statistically inferior flap survival.

Harder et al. developed a mouse model in which a randomized perfused flap is integrated into a back skin chamber [33,34]. This flap developed untreated due to continued ischemia, a partial necrosis of about 50%. Using the intravital fluorescence microscopy it is possible to examine both morphological and dynamic changes in the tissue and the vascular system of the flap repeatedly at the same location in the chamber window [32,33]. A first study investigated the efficacy of recombinant human EPO (RhuEPO: Epoetin b, NeoRecormon; Roche, Basel, Switzerland), which in two different dosages (500 *vs* 5000 ie RhuEPO/kg kg) was first administered 24 hours before flap lifting, i.e. before induction of ischemic stress and following, repeated over 4 days. The administration of RhuEPO showed a significant, dose-dependent anti-inflammatory (i.e. decreased leukocyte-endothelial interaction, restricted Zell apoptose) and pro-angiogenic effect (i.e. microvascular vascular refinement). The lower dosage of 500ie/kg kg resulted in a significantly improved flap survival compared to the untreated animals, whereas the 10 fold higher dosage of 5000ie/kg kg only marginally improved the flap survival. The administration of RhuEPO was able to maintain the perfusion in the capillaries of the critically perfused flap areas. The significant rise of haematocryte, which could only be detected in the high-dose EPO group from the fourth day after first administration, led to a significant deterioration in the flow properties in the lobe and thus to a worse flap survival-Despite rapid EPO-induced anti-inflammatory effect, which was characterized by a reduction in cell apoptosis and leukocyte-endothelial interaction [33].

Having demonstrated that EPO has a dose-dependent protective effect on critically perfused flap tissue, Rezaeian et al. in the same mouse model investigated the optimal timing of EPO administration in relation to flap lifting, i.e. the induction of persistent flap ischemia [34]. For this, 3 x 500 ie RhuEPO/kg kg (rhuEPO: Epoetin B, NeoRecormon; Roche) was administered over a period of 48 hours either before (pre-conditioning) or after (post conditioning) the flap lift. In a third group, the mice received EPO overlapping both 30 minutes before and after flap lifting as well as 24 hours (perioperative treatment) after flap lifting. Both the pre-conditioning and the perioperative treatment led to a significant improvement of the flap survival, which is seen as a result of the maintenance of the capillary perfusion in the critically perfused flap portion. This results from a very early EPO-implanted high-regulation of the Inducable Stickstoffoxid Synthase (INOS), which leads to a dilation of the afferent lobe vessels. If EPO is only administered after induction of iscemia (post conditioning), this iNOS-induced preservation of the lobe perfusion cannot be initiated in time. In addition, a VEGF-transmitted angiogenic reaction was established with a de novo-formation of functional capillaries [34].

In a further work, Rezaeian et al. were also able to show on the mouse model that the persistent vasodilation and thus the improved flap survival after perioperative EPO application is not only INOS mediated, but also by the longer continuous high-regulation of endothelialal Nos (eNOS) [35]. This paper also investigated whether the EPO-induced and VEGF-initiated angiogenesis is actually involved in improved flap survival. The co-administration of RhuEPO and Bevacizumab (Avastin; Roche, Basel, Switzerland), i.e. a VEGF receptor antagonist that acts as an angiogenesis inhibitor, led to a lack of EPO-induced angiogenic response. Interestingly, there was no change in the survival of the flap after EPO administration. The authors concluded that the EPO-transmitted angiogenesis is not involved in the flap survival under these modalities of administration. This is probably due to the time lag, because the newly formed capillaries are only functional 5 days after flap lifting. This time span is beyond the tissue tolerance to iscemia or the demarcation of necrosis [36]. In analogy to microvascular flaps, Contaldo et al. investigated the efficacy of EPO on musculocutaneous tissue, which was subjected to a 3-hour ischemia, followed by a reperfusion (i-/r). 5000ie RhuEPO/kg kg (rhuEPO: Epoetin B, NeoRecormon; Roche) were administered either 1 or 24 hours before ischemia [37]. The animals treated with EPO showed an increased expression of both the EPO receptors in skin and muscle tissue as well as the Nos. These flaps and the animals showed a lower reperfusion injury than untreated animals, which was expressed in a preservation of the capillary perfusion, a decreased hyperpermeability of the vessels and a lower inflammative reaction [37].

In addition, Contaldo et al. investigated in a hamster model of a collateralized island flap, tissue protection after systemic EPO administration (RhuEPO: Epoetin B, NeoRecormon; Roche) [38]. For this purpose, RhuEPO was administered alone or rhuEPO together with a non-specific no-blocker L-nitro-L-arginine methyl ester (L-name). The RhuEPO application led to a significant improvement of the flap perfusion as well as to a weakening of the inflammatory reaction and a decreased rate of apoptosis. Since the co-administration of RhuEPO and L-name led to a complete abolition of tissue protection, the authors concluded that the protective effect is primarily a RhuEPO effect [38].

Large-scale tissue-dissektion and transfers repeatedly lead to

wound healing disorders and partial necrosis of randomized perfused tissue areas.

Accordingly, it would be very desirable to implement the findings gained into the clinic, in particular the application of non-hematocrit-effective EPO dosages or the short-term use of higher EPO dosages before they are problematic hemaocryte-effects. The local EPO application, possibly also high-dosage or alternatively the use of modified non-erythropoietic-effective EPO molecules are of particular importance as well [39].

Peripheral Nervous System

The pro-regenerative effects of EPO in the CNS were among the first questions to be dealt with in this field of research [12,14]. The positive results suggested an investigation into the EPO's effects also in the peripheral nervous system.

The first work dealing with the presence of EPO and EPO receptors in the peripheral nervous system has already been published 2001 [40]. On the one hand, the *in vivo* occurrence of EPO and the EPO hetero receptor in the rat model and on the other the respective *in vitro* occurrence in the cell culture was examined. Both models were evaluated on the one hand in "normal condition" but also after trauma (*in vivo*: chronic constriction, acute bruise trauma or *in vitro*: hypoxia). In this work EPO and EPO hetero receptors have already been described in non-traumatized nerves in the swan cells. Directly post-traumatic, more and more EPO could be detected in neurons as well as in the swan cells; the number of EPO hetero receptors appeared unchanged until slightly degraded. In the EPO (2680ie/kg; Epoietin α, Epogen, Amgen, Thousand Oaks, CA, USA) treated animals and cell cultures a lower number of apoptosis of neurons in motoric ganglions was detected , or in the cells in culture, compared to untreated controls [40].

Another work of the same authors, dealt with further pro-regenerative effects of various EPO dosages (1000ie/kg kg, 2680ie/kg kg, 5000iu/kg kg, 2003). Epoietin *α*, Epogen, Amgen). In this work also a bruising trauma model on the peripheral nerves of the rat was used [41]. A lower rate of apoptosis of the neurons in the motor Ganglion could be shown. In addition, there was a reduction in the time period in which typical pain reactions occurred in the case of the animals treated with EPO. Differences between the different EPO dosages were not described in this work.

Lykissas published 2007 a thesis on the use of EPO (2680ie/kg) in an end-to-side neurorhaphie model in the rat [42]. The aim of the study was to achieve a better germination of the nerves connected end-to-side. This was investigated in a functional way, by analyzing the gait image and the footprints (day 15, 30, 60, 90 and 150), histological probes at the relevant nerves and muscles (day 150), and by means of a weight analysis of the success organs (Tibialis anterior muscle) (day 150). At the first two examination points (Day 15 and 30) of the gait image and footprints, the results of the animals treated with EPO were statistically significantly better compared to the untreated animals. However, after EPO injections no longer occurred, the animals treated with EPO were statistically significantly behind the untreated controls. This difference to the detriment of the animals treated with EPO could be evidenced throughout the entire investigation period. The histological examinations of the nerves and success organs also confirmed the worse functional results, the muscles were statistically significantly stronger atrophied and the histological cuts showed less axone in the nerves. These results could not be explained conclusively by the authors. What remains interesting, however, is the fact that EPO achieved significantly better results in the first few days. Open questions are: On the one hand, the concrete mechanisms that expire after the EPO applications are displaced and on the other, what would happen with a consistent, longer-term EPO application. Referring to the work on neuropathic patients [43] presented below, which EPO received over a period of several months, it would also be possible to achieve a different result if EPO were to be administered over the entire time of the nerve regeneration.

In 2008, Lohmeyer published the first work that specifically focused on the effect of EPO (1000iu/kg kg; RhuEPO: Epoetin B, NeoRecormon; Roche) is engaged in the regeneration of peripheral nerves with a length defect (10mm) after separation in the rat model [44]. Two different reconstruction methods were used: autologous nerve graft and silicone tubes. Seven weeks post-op, the animals were euthanized and the tissues analyzed (histology, muscle weight, Axonanzahl and diameter). Only a significantly lower muscular atrophy could be shown in the groups treated with EPO (P = 0,006). In terms of number of axons and diameter, no differences could be shown in the two groups.

2010 Yin could essentially confirm the results in a rat model [45]. However, statistically significant larger axon diameter, thicker myelin layers and a greater absolute number of axons and improved maturation of the tissue in the EPO group (5000iu/kg kg; RhuEPO: Kirin Brewery, Tokyo, Japan) were described. Also in the functional tests (large and small toe distance as well as nerve conduction speed) the animals of the EPO group were significantly superior to the untreated animals after 8 weeks, whereas after 4 weeks no significant difference could be shown.

In another *in vitro* and mice model, local application of EPO revealed pro regenerative effects. Administration of EPO maintained more *in vivo* myelinated axons at the site of nerve crush injury. *In vitro*, EPO treatment promoted myelin formation and protected myelin from the effects of nitric oxide exposure in co-cultures of Schwann cells and dorsal root ganglion neurons. In a novel, surgically applicable local treatment using Food and Drug Administration-approved fibrin glue as a vehicle, EPO was as effective as systemic EPO administration at time points earlier than those explainable using standard models of neuroregeneration [46].

The only publication known to us on the patient examines EPO effects on the peripheral neuropathy in renal insufficient of prädiatric patients. A significant improvement in the conductivity of the moto neurons was demonstrated after five months of EPO therapy; an effect on the sensory conductivity was not shown [43].

The partly controversial results according to EPO application in the area of regeneration of peripheral nerves require further investigations, in particular regarding application duration and dosage regimes.

Acute Wounds with the Example of Acute Thermal Injuries

As early as 1972, the behavior of autologous EPO values in

patient blood was investigated in anemia after thermal trauma [47]. In particular, it was noted that patients with burns below 30% of KOF usually had normal EPO blood values and were not subject to transfusion. In contrast, in patients with burns over 30% KOF, there is usually a pronounced low level of EPO values in the blood; In addition, these patients were generally anemic. It is also noticeable that patients with pronounced bacterial wound infections had lower EPO and equally pronounced anemia levels and therefore all patients in these two groups were subject to transfusion. In this study, only anemia or the need for transfusions was evaluated [47].

Since the 1980s, multiple reports on the use of EPO in thermally injured patients have been found to reduce or eliminate the number of transfused erythrocyte concentrates. None of the publications showed a significant reduction in the number of transfused blood products needed; an effective increase of the erythropoiesis, which had been intended, could not be shown as well. Effects on wound healing are not described since they were not content of the investigations [48,49].

In 2006, Galleano published the first work examining the pro-regenerative effects of RhEPO after thermal trauma in the mouse model [50]. Three groups were formed: 50 animals in toto the verum group received: RhEPO 400IE/kg kg/d for 14 days, in the placebo group: distilled water or in the control group: They were in advance RhEPO immunized and received RhEPO 400IE/kg/d for 14 Days (no indication of the product used in the original work). This showed a significantly faster wound closure combined with a faster re-Epithelialisierung in the verum group compared to the other two groups. Compared to the placebo group (destilled water), the wound healing in the immunised group (control) was once again significantly delayed. The verum group showed, in the respective comparison, a significantly better epithelial proliferation, a much more mature extrazellular matrix and a forced angiogenesis. This was shown in particular by the higher microvascular density in the histological cuts, which also had a corresponding, elevated CD31 expression, as well as increased VEGF expression. The blood-image changes were not statistically significant, but the day 14 showed slightly elevated erythrocyte and reticulocytes values for the animals treated with EPO [50].

Comparable to the perfusing promoting effects in iscemic flaps, Tobalem could describe prevention of secondary burn progression, prevention of subdermal-plexus thrombosis, and NO release optimizing tissue perfusion, as well as its anti-apoptotic effects due to EPO application onto a standarized burn model in rats [51].

The effect of topical applied EPO was investigated in a mouse model, in standardized water vapor scalds (RhuEPO: Epoetin B, NeoRecormon; Roche, Basel, Switzerland). This showed that the deep-dermal scalds in the animals treated with EPO-Hydrogel were cured much faster; even the re-epithelialization was completed earlier. Likewise, increased epithelial proliferation, a faster formation and maturation of the extracellular matrix as well as an induction of angiogenesis and resulting higher capillary densities, which could also be demonstrated by high CD31, VEGF and ENOS levels [52]. In a further work on the mouse model, the combined presence of EPOR and the EPO hetero receptor could be demonstrated in both the healthy and the scalded mice skin. In the healthy skin, a significant reduction of the EPOR-expression according to EPO application could be evidenced, not in the thermally injured, here the expression rate remains high. Likewise, the faster and higher quality wound healing (dermis/Epidermis papillon, maturity of the extracellular matrix) could be shown under systemic and topical EPO application [53,54].

A pilot study on topical application was carried out on 11 burned patients. In this project, the local EPO-Hydrogel (RhuEPO: Epoetin B, NeoRecormon; Roche, Basel, Switzerland) or used in the controls placebo-Hydrogel. A significantly faster healing of the split skin donor sites in the verum group could be observed. Thus, after 7 days at 85% of patients, a complete cure of the EPO treated split skin donor sites was observed [55].

In order to verify the promising results of the preclinical investigations clinical trial was initiated, funded by the Federal Ministry of Education and Research, wich investigates systemic applied low-dose EPO in burned patients (150ie, RhuEPO: Epoetin b, NeoRecormon; Roche, Basel, Switzerland) (EPO in Burns, Eura CT number: 2006-002886-38, protocol number: 0506, ISRCT Number: ISRCTN95777824) [56].

Chronic Wounds

Another interesting potential may also be the treatment of chronic wounds. In particular, sole or supporting minimally invasive therapy options are an important field of research to replace extended plastic-surgical reconstruction techniques (e.g. free microvascular flaps). Patients suffering from chronic wounds are often not adequately assisted with previous conservative measures, so unfortunately there are still too many (major) amputations. [57].

Previously published work, for example, wound healing has been evaluated in ischemic edges of the skin wounds of the rat [55]. 84 animals were divided into four groups of 21 animals, two groups were given a minor ischemic incision and two were marked ischemic. One of the groups received verum (400ie/kg EPO subcutaneous for 10 days, no information about the product used in original work) the other two served as a control and received the injection solution without EPO according to the same scheme. After 3, 5 and 10 days Hemoglobin (HB) and red blood cell numbers were determined, and in both verum groups after 10 days increased values were shown. Immunohistochemical dyes (micro vessel density), hydroxyproline and VEGF regulations were carried out after 3, 5 and 10 days from the wound areas. Day 3 and 5 showed a statistically larger microvascular density and also the highest VEGF values in the EPO-treated animals, especially in the pronounced ischemic wounds. On day 10 no differences were to be seen. The hydroxyproline content was generally lower in the pronounced ischemic wounds than in the minor ischemic, but on day 5 and 10 showed significantly higher values in the EPO treated animals than in the controls [58]. This work confirms the close correlation between EPO and VEGF and shows the importance for a fast and effective angiogenesis in the wound area.

Further work has investigated wound healing on the diabetic mouse model (400IE/kg EPO intraperitoneal application of EPO for 12 days or carrier solution as control) [59]. A total of 84 animals were divided into 4 groups (21 animals; 2 Verum, 2 control groups), 42 animals were hyperglycemic, 42 not, 7 animals each were euthanized

after 3, 6 or 12 days. After 3, 6 and 12 days HB and number of red blood cell counts were determined, and in both verum groups after 12 days showed slightly elevated values. The wound areas were examined in each case for VEGF and CD31 content, histological organization and maturity as well as the mechanical stability (on day 12). Above all, increased VEGF and CD31 values were shown in the EPO groups, resulting in higher capillary densities than in the control groups. This is further underlined by a higher CD31 gene expression after 3 and 6 days. On day 12, the respective values are readjusted. The histological maturity and organization was already more mature in the EPO groups after 6 days than in the controls. The mechanical stability of the young scars was significantly greater after 12 days in the EPO groups than in the controls, with statistically significant improvements of the values in both normoglycemic and diabetic animals [59].

Hamed could describe in 2010 in a diabetic rat model a dose dependent significantly improved wound healing in the full-layer wound model with topical EPO therapy (600 IE and 3000ie EPO/g creme 1xd for 12 days; Darbepoietin α, ARANESP, Amgen AG, Zug, Switzerland) [60]. This showed a significant reduction in the healing time, a significantly increased microvascular density and increased VEGF and hydroxyl proline values in the EPO-treated groups compared to the control group. The highly dosed group treated with EPO showed better results than the group treated with low EPO dosages. A rise in hematocrite or other systemic EPO effects could not be evidenced [60].

Also other *in vitro* experiments confirmed the effect of EPO on dermal mesenchymal stem cells from human skin that were cultivated under hypoxia conditions. In addition, increased concentrations of IL-6 were added to the culture medium, which alone significantly inhibited proliferation. EPO (RhuEPO: Epoetin B, NeoRecormon; Roche, Basel, Switzerland) in the cultural medium, however, showed an increased proliferation rate [61].

The use EPO in problem wounds in humans has been described so far by two publications, a case report and a publication that represents four individual healing attempts.

Ferri et al 2010 described the treatment of a scleroderma patient with low dose EPO S.C. for a total of six months with degrading dosage (no manufacturer and Präparat angaben in original work) [62]. On the one side, the ulcers themselves were investigated as well as the need for analgetic therapy was assessed, expected adverse effects, such as blood image changes evaluated and bone marrow punctures were taken before and after therapy. The samples were studied on Endothelial Progenitor Cells (EPCs) and studied in more detail on their biological state. The ulcers were cured within six months, while the patient remained without a recurrence during the 12-month follow-up period. The pain-relieving requirement could be significantly reduced within two months. Significant changes in the blood image could not be shown. Compared to controls of healthy test subjects (n =7), the analyses of bown marrow biopsies taken prior to therapy showed reduced numbers of EPC stem cell-typically marked cells, the number of apoptotic EPCs was also higher. After six months of EPO treatment, the picture was clearly changed: the number of cells marked by EPC had risen statistically significantly and the rate of apoptosis decreased [62].

The publication of Keast and Fraser from the year 2004 describes the treatment of four anemic and paraplegic patients with chronic sores with systemic EPO (75IU three times weekly SC, no manufacturer and Präparat angaben in original work) about a six-week period. In all patients a significant reduction in the size of the ulcers, partly the complete remission could be achieved. In addition, a clear general increase in performance and the decline of chronic infections were described [63].

Discussion and Conclusions

The results of the tests presented on ischemic flap models, peripheral nerves, chronic wounds and thermal injuries are sufficiently promising and should lead to the implementation of further pre-clinical and clinical studies to investigate the possibilities of EPO as a therapeutic agent in the field of treatment optimization through targeted induced pro-regenerative effects on wound healing.

To sum up, EPO can reduce both damage to the flap model caused by acute persistent ischemia as well as ischemia/reperfusions damage. This shows both a time dependence on the flap lift (ischemia induction), as well as a dose dependence on EPO. A significant increase in hematocrite, as seen in these models after repeated administration of high-dose EPO, can worsen the flow characteristics of the blood to thromboembolic complications [64] and so the EPO-transmitted protective, anti-ischemic effects. On the other hand, for example, in a mouse model [65], but especially in humans, an anti-thrombotic effect of EPO applications could already be shown, so that the scientific discussion is by no means completed [66]. The tissue protective effect of EPO appears to be in the ischemic tissue, e.g. flap models, primarily No-associated, in which it can maintain the perfusion in the critically perfused flap (-areal), especially in the case of a halting ischemia. The angiogenesis does not seem to play the decisive role, since the newly formed vessels are only functional after about 5 days, a time when the necrosis of the flap has already been irreversibly demarked.

From today's perspective, the work of Campana and Myers can be seen critically and a repetition of the experiments with newly developed, highly-specific antibodies against the EPO hetero receptor would be desirable. Nevertheless, the functional results shown here, albeit controversial, are largely encouraging and allow EPO to be effective in regenerating peripheral nerves. Noteworthy are the effects that were demonstrable, for example, on the success organs and could explain why the functional results in the EPO-treated animals were, for example, significantly better. In the human case reports, the nerve conduction speed was measured, which showed better results for the patients treated with EPO than for the untreated. Seen in this way, a direct effect on the peripheral nerves can be assumed. This assumption is also supported by the histological results of Yin [45]. Unresolved questions are still the optimal dosage and the most effective application period.

An important aspect is the described resistence to the erytropoietic effects of EPO in thermally injured patients. However, even more import and might be the very good survival figures of the treated patients. The results of the pilot study with local applied EPO are interesting, but must be further investigated in GCP-conform clinical trials [48,55].

Even in the treatment of chronic wounds of different genesis, EPO appears to be a possible new candidate. Open questions in this area should be in particular the dosage and duration of the therapy. Particularly impressive are the healing successes shown in the case reports, which however also require verification in clinical trials [60,62,63].

In the discussed work, EPO presents itself as an interesting pharmaceutical product in the treatment of various typical plastic-surgical questions and problems. A possible future expansion of the range of indications for the use of EPO to the typical described problem areas appears, according to current knowledge, quite desirable.

Since EPO is no longer able to obtain patent protection in its classic dosage form as an injectable solution, it is to be feared that the interest in the required clinical studies by industry is considered to be low, the research and funding institutions of the public authorities are therefore particularly required.

However, for a possible individual use of EPO in the plastic surgical patient collectives, a very careful patient evaluation must be advised. Especially with regard to problematic pre-conditions such as hypertension, thrombembolische events or malignancies, a particularly careful anamnesis and consideration of the risk-benefit ratio must be advised [67].

A possible alternative would be the further development and testing of non-hematopoetic-effective EPO derivatives. However, a longer period of time is still to be expected, until they have taken the hurdle of the necessary admission studies and other preconditions and are available for widespread clinical use [20,39].

In this area of research, there is definitely lots of room for future investigations.

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