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EXPERIMENTAL RESEARCH - VASCULAR

Levosimendan, a new therapeutic approach to prevent delayed cerebral vasospasm after subarachnoid hemorrhage?

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Abstract
Background Under physiological cerebral conditions, levosimendan, a calcium-channel sensitizer, has a dose-dependent antagonistic effect on prostaglandin F2alpha (PGF)-induced vasoconstriction. This circumstance could be used in antagonizing delayed cerebral vasospasm (dCVS), one of the main complications after subarachnoid hemorrhage (SAH), leading to delayed cerebral ischemia and ischemic neurological deficits. Data already exist that identified neuroprotective effects of levosimendan in a traumatic brain injury model and additionally, it has been proven that this compound prevents narrowing of the basilar artery (BA) luminal area after SAH in an in vitro rabbit model. Takotsubo cardiomyopathy, a severe ventricular dysfunction, is also a well-known complication after SAH, associated with pulmonary edema and prolonged intubation.
Methods The polypeptide endothelin-1 (ET-1) plays a key role in the development of dCVS after SAH. Therefore, the aim of the present investigation was to detect functional interactions between the calcium-sensitizing and the ET-1-dependent vasoconstriction after experimental-induced SAH; interactions between levosimendan and a substrate-specific vasorelaxation in the BA were also examined. It was reviewed whether levosimendan has a beneficial influence on endothelin(A) and/or endothelin(B_1) receptors (ET-(A) and ET-(B_1) receptors) in cerebral vessels after SAH. We also examined whether this drug could have antagonistic effects on a PGF-induced vasoconstriction.
Results Under treatment with levosimendan after SAH, the endothelin system seems to be affected. The ET-1-induced contraction is decreased, not significantly. In addition, we detected changes in the nitric oxide-cyclic guanosine monophosphate (NO-cGMP) pathway. Preincubation with levosimendan causes a modulatory effect on the ET-(B_1) receptor-dependent vasorelaxation. It induces an upregulation of the NO-cGMP pathway with a significantly increased relaxation. Even after PGF-induced precontraction a dose-dependent relaxation was registered, which was significantly higher (E_{max}) and earlier (pD_2) compared to the concentration–effect curve without levosimendan.
Conclusions After experimental-induced dCVS, levosimendan seems to restore the well-known impaired function of the vasorelaxant ET-(B_1) receptor. Levosimendan also reversed the PGF-induced contraction dose-dependently. Both of these mechanisms could be used for antagonizing dCVS in patients suffering SAH. Levosimendan could even be used additionally in treating patients developing takotsubo cardiomyopathy.

Keywords Levosimendan · dCVS · Delayed cerebral vasospasm · Takotsubo cardiomyopathy · Subarachnoid hemorrhage · SAH · ET-(A) receptor · ET-(B_1) receptor · ET-1 · Endothelin · Prostaglandin F2alpha · PGF

Introduction

For decades, intensive research has been conducted to improve the outcome of patients suffering subarachnoid hemorrhage (SAH), which is decisively influenced by two events:

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one by the rupture of an intracranial aneurysm resulting in increased intracranial pressure, and the other by delayed cerebral vasospasm (dCVS). Since mortality due to aneurysm rerupture could be decreased by development of microsurgical clipping techniques and endovascular coiling, dCVS remains one of the main causes contributing to the poor outcome [16, 17, 41]. Some patients suffering SAH develop cardiological complications: takotsubo cardiomyopathy, a severe transient left-ventricular dysfunction, is a well-known complication [26, 27, 40]. Meanwhile, a multifactorial process has been identified, including cerebral inflammation [6, 31], early brain injury [9, 35], cortical spreading depression [7], and loss of the pressure-dependent cerebral autoregulation [4] as being responsible for the poor overall outcome (Table 1).

One of the main causes for developing dCVS seems to be endothelin-1 (ET-1), a potent and long-lasting vasoconstrictor, which increases the cerebrospinal fluid after SAH [34, 37, 44]. In the cerebrovasculature, ET-1 acts over two specific receptors, the endothelin(A) and endothelin(B_1) receptor (ET-(A) and ET-(B_1) receptor). While in smooth muscle cells, the ET-(A) receptor is localized, inducing vasoconstriction, the ET-(B_1) receptor is located in the endothelium, mediating vasodilatation via the nitric oxide-cyclic guanosine monophosphate (NO-cGMP) pathway, which is diminished after SAH [11, 19, 22, 28, 36, 42, 45].

Levosimendan, a novel calcium-channel sensitizer, is widely established in the treatment of ICU patients with congestive heart failure [2, 8]. In the treatment of takotsubo cardiomyopathy, a cardiac disease that contraindicates the use of catecholamine inotropes, there is literature that shows beneficial effects of using levosimendan [1, 29, 35], data even exist that show that sepsis-associated takotsubo cardiopathy could be reversed [15]. There are also a lot of data that detected neuroprotective effects of levosimendan without affecting the brain metabolism in a traumatic brain injury model [32, 33], beneficial effects in preventing inflammation [12, 14, 18, 25] and a positive affection of the cerebral autoregulation [12]. While no data for a beneficial effect on cortical spreading depression exist, our own investigation already showed under physiological conditions a possible positive effect against dCVS by preventing prostaglandin F2alpha (PGF)-induced vasocontraction [21]. Despite its effect on early brain injury, inflammation, and cerebral autoregulation, levosimendan has not yet been tested for further implications after experimental-induced SAH; particularly therefore we analyzed the possible interactions of levosimendan on cerebral arteries in a pathophysiological setup.

Materials and methods

General

All procedures proceeded in compliance with German Federal Guidelines for animal experiments and with the permission of the local ethics committee.

Table 1 Vasorelaxing effects in basilar artery ring segments. All values are expressed as median ± percentiles

	Relaxation after PGF precontraction	$E_{(max)}$	pD_2	*n*
Solvent control SAH		24 % ± 20 %/30 %	3.99 ± 4.18/3.84	5
Levosimendan SAH		61 % ± 55 %/66 %#	5.14 ± 5.85/4.55#	5
# $p < 0.01$				
	S6c			
Solvent control SAH		20 % ± 14 %/27 %	11.56 ± 11.65/11.43	5
Levosimendan				
10-4 M SAH		56 % ± 45 %/59 %%*	12.41 ± 13.05/11.00	5
* $p < 0.05$				
	ACH			
Solvent control SAH		83 % ± 79 %/84 %	6.58 ± 6.86/6.51	5
Levosimendan 10-4 M SAH		87 % ± 86 %/89 %	7.41 ± 7.45/7.08	5
	SNP			
Solvent control SAH		51 % ± 46 %/57 %	4.68 ± 5.09/4.51	6
Levosimendan 10-4 M SAH		86 % ± 76 %/89 %#	5.95 ± 6.05/5.85#	6
# $p < 0.01$				
	8Br-cGMP			
Solvent control SAH		26 % ± 17 %/33 %	5.07 ± 5.92/4.79	6
Levosimendan 10-4 M SAH		52 % ± 44 %/60 %*	6.23 ± 6.52/6.08	6
* $p < 0.05$				

Basal conditions

By injection of autologous blood into the cisterna magna, experimental-induced SAH was performed on days 1 and 2, using an adapted double-hemorrhage-model as described previously [10, 45]. On day 5, where, as angiographically has already shown, a maximum of dCVS occurs [45], male Sprague–Dawley rats, each weighing between 250 and 400 g, were anesthetized with CO_2 and then sacrificed by exsanguination after cutting the external and internal carotid artery. The brain, along with the cerebral vessels, was excised and immersed in a cold modified Krebs–Högestätt solution containing the following components (in mM): NaCl, 119; KCl, 3.0; NaH_2PO_4, 1.2; $CaCl_2$, 1.5; $MgCl_2$, 1.2; $NaHCO_3$, 15; and glucose, 10 (Figs. 1 and 2).

The basilar artery (BA) was carefully dissected from the brainstem by using a binocular microscope and cut into four equal parts, each approximately 2 mm in length (Fig. 3). These BA ring segments were meticulously mounted on L-shaped stainless-steel rods in an organ bath (IOA-5301; FMI GmbH) for measurement of isometric force, as described earlier in detail [23, 42]. Special care was taken to avoid any damage to the intimal surface. The isometric force was determined by a transducer (GM Scaime, Annemasse Cedex, France) and recorded digitally.

Organ baths were filled with modified Krebs–Högestätt solution and continuously bubbled with a humidified gas mixture (95 % O_2, 5 % CO_2), resulting in a pH of approximately 7.35. For the investigation, 60 segments were used for analysis; 19 were excluded. Every ring segment has been taken just for one single measurement of isometric force.

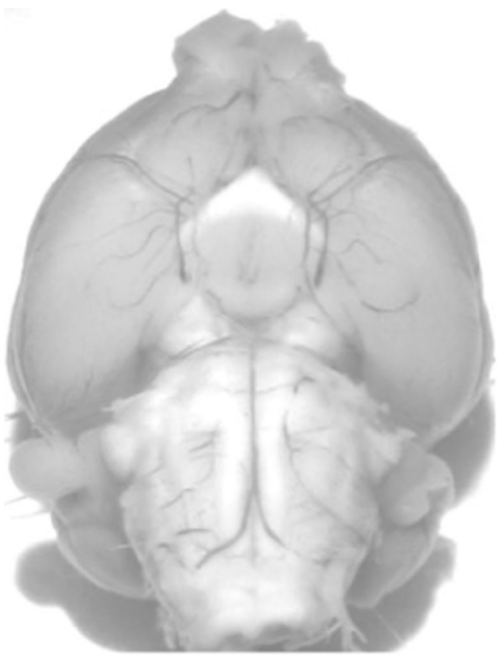

Fig. 1 Prepared rat brain with cerebral vessels immersed in a cold modified Krebs–Högestätt solution

After the BA ring segments were mounted, the temperature in the organ baths was gradually increased to 37 °C. During the adaptation period, the vessels were adjusted repeatedly to a stable resting tension of 3.3 milliNewton (mN).

Validation procedures and variables measured

At the end of the adaptation period, a reference contraction was induced by 124 mM potassium + Krebs solution (124 mM KCl) (Krebs–Högestätt solution with equimolar exchange of NaCl by KCl), which was repeated at the end of the experiments (Fig. 4a). Segments with less than 2 mN contractions were excluded from the experiment, as well as those developing less than 75 % of the first reference contraction at the end of the experiment. The functional integrity of the endothelium was tested by administration of acetylcholine (ACH; 10^{-4} M) after precontraction with 5-hydroxytryptamine (5-HT; 10^{-5} M). A relaxation of more than 30 % of the precontraction indicated a functionally intact endothelium (Fig. 4b). Segments with endothelial dysfunction were also excluded from further assessment. Contraction was measured in mN force and expressed as a percentage of reference contraction.

Levosimendan (10^{-4} M) was added in the organ bath and compared to a solvent control group. In each case, only one investigation (with or without levosimendan) a concentration–effect curve (CEC) was performed by cumulative application of ET-1 to avoid tachyphylaxis.

After precontraction by PGF, the vasorelaxant effect of levosimendan was tested by cumulative application and compared to a solvent control group.

Therefore, concentration–effect curves were performed for several compounds in the presence and absence of levosimendan.

Compounds and solvents

All compounds, except Levosimendan, were freshly dissolved in distilled water on the day of the experiment. Levosimendan was dissolved in dimethyl sulfoxide. The compounds used are shown below.

PGF: a potent vasoconstrictor, acting over a specific receptor located on smooth muscle cells. Purchased from Sigma-Aldrich (Schnelldorf, Germany).

ET-1: a potent and long-lasting vasoconstrictor, acting over an ET-(A) receptor located on smooth muscle cells. Also purchased from Sigma-Aldrich (Schnelldorf, Germany).

5-HT: a potent vasoconstrictive neurotransmitter. Also purchased from Sigma-Aldrich (Schnelldorf, Germany).

ACH: a vasorelaxant neurotransmitter, acting over specific receptors on endothelium. Also purchased from Sigma-Aldrich (Schnelldorf, Germany).

Fig. 2 Microinstruments used for microscopic dissection of the basilar artery from the brain stem

S6c: a vasorelaxant peptide, acting over a ET-(B_1) receptor located on the endothelium. Purchased from Calbiochem-Novabiochem (Bad Soden, Germany).

SNP: a vasorelaxant peptide. Purchased from Enzo Life Sciences GmbH (Lörrach, Germany).

8Br-cGMP: a cGMP analogue. Purchased from Enzo Life Sciences GmbH (Lörrach, Germany).

Levosimendan: a calcium-channel sensitizer. Kindly provided by Jouko Levijoki, M. Sc. (Pharm.) from Orion Corporation, Orion Pharma (Espoo, Finland).

Analysis of results

Contraction was measured in mN force and given as a percentage of reference contraction. Relaxation was calculated as a percentage-decrease of the corresponding precontraction induced by 5-HT, PGF, or 62 mM KCl. All values in the text and figures are given as median ± percentiles.

For each completed CEC, the maximum contraction (E_{max}) and pD_2 ($-\log 10EC_{50}$) or EC_{50} (i.e., the concentration at which half of the maximal effect occurs) were calculated. The pD_2 was calculated by linear regression analysis of the CEC, after logarithmic transformation of the concentrations above and below the EC_{50}.

All statistical analyses were planned and performed using the non-parametric Wilcoxon–Mann–Whitney *U* test.

A probability value (*p*) less than 0.05 was considered as significant.

Results

Effect of levosimendan on basal tone or 124 mM KCl-induced contraction on basilar artery ring segments Levosimendan changed neither the basal tone nor the 124 mM KCl-induced contraction. The contraction of the segments was expressed as a percentage of the contraction induced by the first 124 mM potassium + Krebs solution.

Effect of levosimendan on the ET-(A) receptor-induced vasocontraction on basilar artery ring segments ET-1 induced a dose-dependent vasocontraction, which is mediated by the ET-(A) receptor [28]. This contraction was induced in the levosimendan and the solvent control group (Fig. 5). After SAH, the loss of ET-(B_1) receptor-dependent vasorelaxation results in an enhanced dose-dependent vasoconstriction to ET-1 [22]. After treatment with levosimendan, the ET-1 induced contraction was non-significantly decreased. The pD_2 was not changed compared to the solvent control group. Under levosimendan 10^{-4} M SAH, the maximum contraction E_{max} was non-significantly lowered (116 % ± 109 %/117 %) compared to solvent control SAH (129 % ± 127 %/133 %). The contraction of the segments was expressed as a percentage of the contraction induced by the first 124 mM potassium + Krebs solution. Values are expressed as median ± percentiles.

Effect of levosimendan on prostaglandin F2alpha-induced contraction on basilar artery ring segments Under

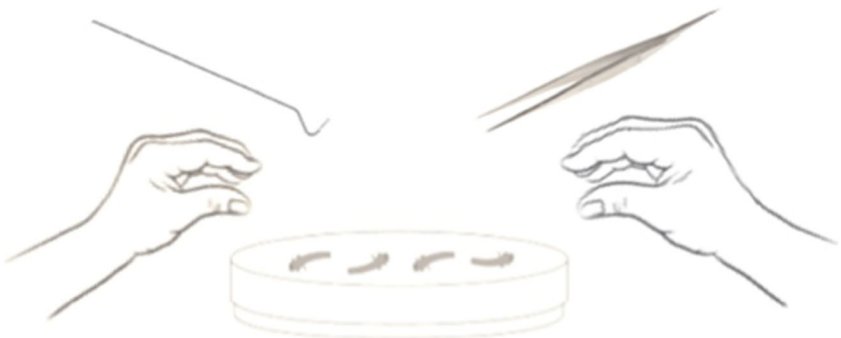

Fig. 3 Under microscopic view, the basilar artery was carefully cut into four equal parts. Ring segments were meticulously mounted on L-shaped stainless-steel rods, afterwards on a fixed L-shaped stainless-steel rod in an organ bath (IOA-5301; FMI GmbH)

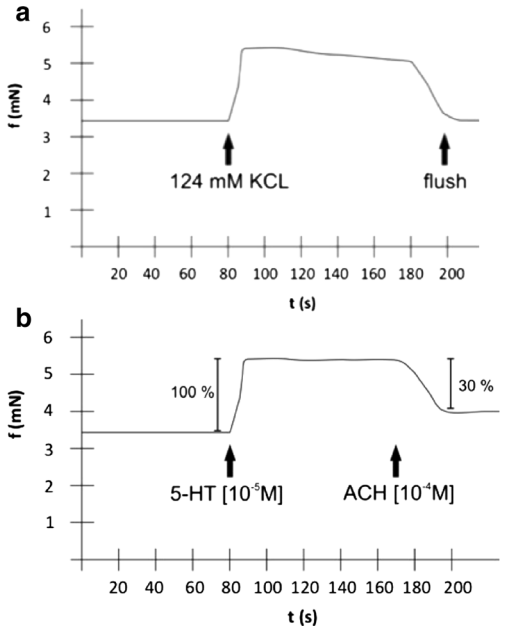

Fig. 4 Isometric force was measured by a transducer (GM Scaime, Annemasse Cedex, France) and recorded digitally. **a** Reference contraction induced by 124 mM potassium + Krebs solution. **b** Functional integrity of the endothelium tested by administration of acetylcholine (ACH; 10^{-4} M) after precontraction with 5-hydroxytryptamine (5-HT; 10^{-5} M). A relaxation of more than 30 % of the precontraction indicated a functionally intact endothelium

physiological conditions, PGF-induced contraction was reversed by levosimendan [21]; and it is already known that in patients suffering dCVS, after SAH, PGF levels are increased [3]. In our investigation, after experimental-induced dCVS, PGF-induced vasocontraction was dose-dependently relaxed by levosimendan. A significant increased relaxation

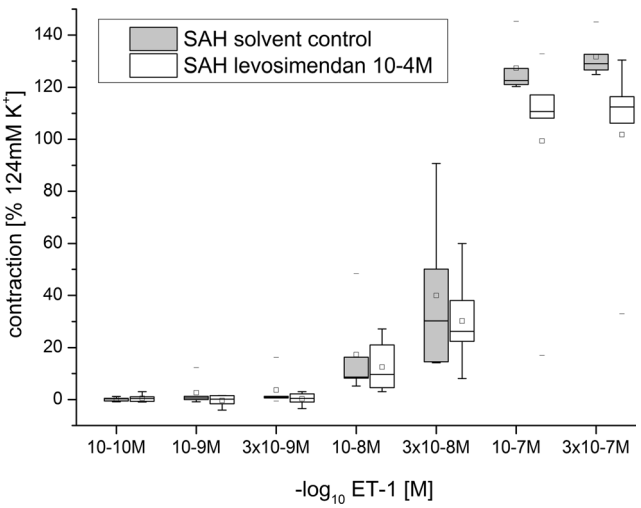

Fig. 5 Constrictive effect of endothelin-1 on the basilar artery tone under levosimendan. Application of endothelin-1 induced dose-dependent vasocontraction in both groups. Adding levosimendan before ET-1 resulted in a reduced E_{max} and a comparable pD_2. The contraction of the segments was expressed as a percentage of the contraction induced by the first 124 mM KCL. Values are expressed as median ± percentiles

E_{max} and enhanced sensitivity (pD_2) were calculated compared to the solvent group (Fig. 6).

Effect of levosimendan on the ET-(B_1) receptor-dependent pathway To investigate the vasorelaxation, segments were precontracted with 62 mM KCL. S6c, a selective ET-(B_1) receptor agonist, was tested after preincubation with and without levosimendan. After experimental-induced SAH, S6c alone did not induce a dose-dependent relaxation. After preincubation with levosimendan, the relaxation to S6c was significantly enhanced (E_{max}) without changes of the sensitivity (pD_2) (Fig. 7a).

ACH induced dose-dependent relaxation in all groups. Preincubation with levosimendan resulted in a significantly increased E_{max} for ACH 10^{-7} and 10^{-4} M (Fig. 7b).

SNP caused a concentration-dependent relaxation of BA ring segments. For the levosimendan group, a higher sensitivity was calculated and the vasorelaxation (E_{max}) was significantly increased (Fig. 7c).

8Br-cGMP, a cGMP analogue, induced dose-dependent relaxation in both groups. For the levosimendan group, a significantly higher vasorelaxation was calculated (Fig. 7d).

Discussion

Our investigations demonstrate the changes of the cerebrovascular contractility after experimental induced SAH by

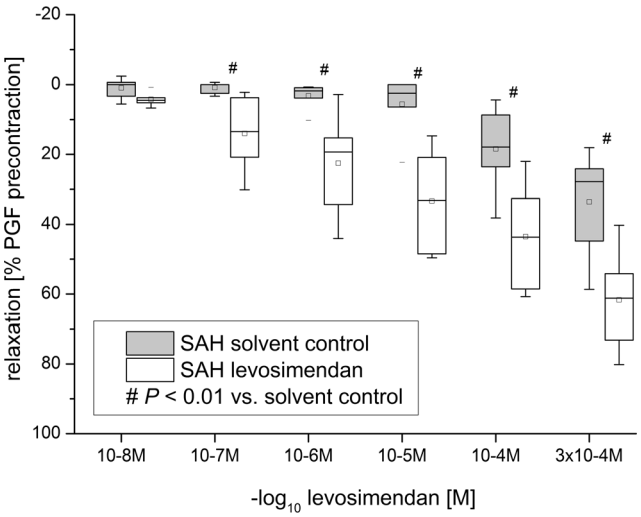

Fig. 6 Vasorelaxing effect of levosimendan in prostaglandin F2alpha-precontracted segments. Levosimendan induced a dose-dependent relaxation with significant higher E_{max} and significant earlier (pD_2) relaxations compared to the group without levosimendan (solvent control). In this group, a spontaneous relaxation of the precontracted vessels was observed. The relaxation of the segments was expressed as a percentage of the contraction induced by PGF 10-5 M. Values are expressed as median ± percentiles. Rhomb (#; $p < 0.01$) denotes statistical significance compared to the solvent control group

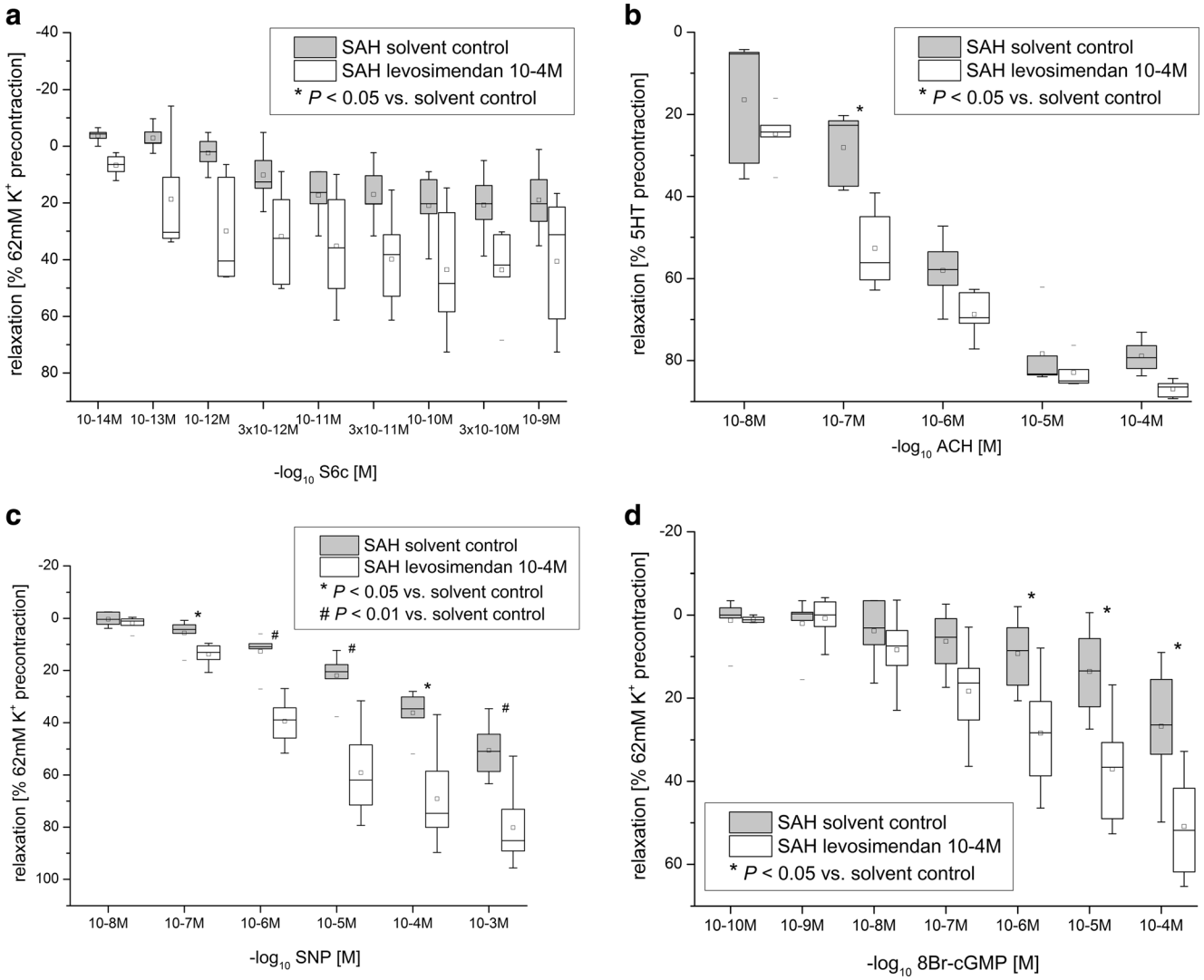

Fig. 7 **a** Vasorelaxing effect of the ET-(B_1) receptor agonist sarafotoxin S6c under levosimendan. After precontraction by 62 mM KCl solution and after preincubation with or without levosimendan, sarafotoxin S6c induced dose-dependent relaxation. The vasorelaxing effect was significantly higher in the levosimendan group. Under levosimendan, the relaxation was significantly enhanced (E_{max}); also a trend towards an earlier relaxation (pD_2) was calculated. The relaxation of the segments was expressed as a percentage of the contraction induced by 62 mM KCl solution. Values are expressed as median ± percentiles. **b** Vasorelaxing effect of acetylcholine under levosimendan. Levosimendan induced a dose-dependent relaxation with a significantly higher E_{max} only at the concentrations ACH 10–7 M and 10-4 M compared to the group without levosimendan (solvent control). The relaxation of the segments was expressed as a percentage of the contraction induced by PGF 10-5 M. Values are expressed as median ± percentiles. *Rhomb* (#; $p < 0.01$) and *asterisk* (*; $p < 0.05$) denote statistical significance compared to the solvent control group. **c** Vasorelaxing effect due SNP after preincubation with levosimendan. After precontraction by 62 mM KCl solution and after preincubation with or without levosimendan, SNP induced dose-dependent vasorelaxation. Shown are the CECs in the presence and absence of levosimendan. The vasorelaxing effect for nearly all concentrations was significantly higher in the levosimendan group, statistically significant for the (E_{max}). Furthermore, the pD_2 was significantly shifted to the left. The relaxation of the segments was expressed as a percentage of the contraction induced by 62 mM KCl. Values are expressed as median ± percentiles. The *asterisk* (*; $p < 0.05$) and *rhomb* (#; $p < 0.01$) denote statistical significance compared to the solvent control group. **d** Vasorelaxing effect due 8Br-cGMP after preincubation with levosimendan. After precontraction by 62 mM KCl solution and after preincubation with or without levosimendan, 8Br-cGMP also induced dose-dependent relaxation. Shown are the CECs in the presence and absence of levosimendan. The vasorelaxing effect was significantly higher in the levosimendan group. Under levosimendan treatment, the relaxation (E_{max}) was significantly enhanced, and a trend towards an earlier relaxation (pD_2) was calculated. The relaxation of the segments was expressed as a percentage of the contraction induced by 62 mM KCl solution. Values are expressed as median ± percentiles. The *asterisk* (*; $p < 0.05$) denotes statistical significance compared to the solvent control group

prior preincubation with levosimendan. To our knowledge, the present study is the first report to demonstrate beneficial functional effects of levosimendan in the cerebrovasculature after experimental-induced dCVS.

Effect of levosimendan on the ET-(A) receptor-induced contraction and ET-(B_1) receptor-induced relaxation

In cerebral arteries, under physiological conditions, an effect mediated by levosimendan to the endothelin system was not detected [21]. After experimental SAH under levosimendan, the ET-1-induced vasoconstriction was decreased in a non-significant way, whereas typically an enhanced contraction was observed [41] (Fig. 5). The S6c-induced vasorelaxation was enhanced in a significant way ($p < 0.05$; Fig. 7a). Other functional investigations confirm this result by an also significantly enhanced vasodilatation of the whole ET-(B_1) receptor pathway (SNP und 8Br-cGMP; Fig. 7c, d). SNP, a direct NO donor, shows significant earlier and higher relaxation in the presence of levosimendan, which seems to be reasonable due to the known loss of ET-(B_1) receptor function, but preserved receptors [43]. These facts indicate that in pathological conditions, levosimendan seems to influence the ET-(B_1) receptor (enhanced relaxation) and potentially the ET-(A) receptor (reduced contraction). Considering this, it is possible that the reason for the non-significantly reduced ET-1 contraction after preincubation with levosimendan (Fig. 5) could be additionally explained by a reduced ET-1 contraction or a reduced ET-(A) receptor activation. An investigation of whether there is a specific contraction-preventing effect from levosimendan on the ET-(A) receptor could be done by preincubation with BQ-788, a selective ET-(B_1) receptor antagonist and BQ-123, a selective ET-(A) receptor antagonist.

Under physiological conditions, neither an ET-(A) receptor-dependent nor an ET-(B_1) receptor-dependent effect was detectable [21]. After induced dCVS by SAH, the ET-1- and S6c-dependent pathways were altered (Figs. 5 and 7a) and exactly this effect seems to be endothelium-dependent. After SAH, the ET-(B_1) receptor relaxation is impaired, resulting in a higher and earlier ET-1-dependent vasocontraction [42, 43] leading to dCVS. However, after levosimendan treatment, this loss of ET-(B_1) receptor function could be partly reversed (Fig. 7a). S6c causes, as already mentioned, significantly increased relaxation in the presence of levosimendan. Therefore, the present data suggest that levosimendan has a positive modulatory effect on the ET-(B_1) receptor. Activation of the ET-(B_1) receptor causes an increased relaxation via the NO-cGMP pathway.

Effect of levosimendan on PGF-dependent vasocontraction

Physiologically and after experimental-induced dCVS, levosimendan induced a dose-dependent relaxation (Fig. 6). After SAH, an imbalance of the PGF–prostacyclin–thromboxane system seems to be one part of the multifactorial process resulting in cerebral inflammation and dCVS [38]. In a pilot study, dCVS after SAH was treated successfully by a prostacyclin infusion [24].

In addition to inflammatory changes, the relaxant effect of levosimendan may be another reason for the positive effects in brain injury models and after SAH [13, 32], therefore more studies are required to examine inflammatory changes of the cerebrovasculature by levosimendan treatment during experimental SAH.

Levosimendan in pathological conditions and further implications

After experimental-induced dCVS, levosimendan seems to have positive effects on the prostaglandin and endothelin system, which plays a key role in the development of dCVS after SAH [22, 37, 41]. The endothelin system acts over two receptors: a contractile ET-(A) receptor, located in the muscular media, and a vasodilatative-acting ET-(B_1) receptor, located in the endothelium. A contractile FP receptor is located in the muscular media and arachidonic acids are constantly synthesized in the endothelium. Normally a certain balance between the vasocontractile and -dilatative pathway exists. After SAH, the ET-(B_1) receptor-mediated vasorelaxing pathway is impaired, which leads to dCVS. Through the elevated PGF synthesis, the vasoconstriction is enhanced. In addition, one finds an elevated ET-1 synthesis after SAH [37]. For the ET-(B_1) receptor, a positive effect of levosimendan could be determined: the well-known impaired ET-(B_1)-vasorelaxation after SAH is partly reversed, resulting in a higher vasorelaxation. These observations, which have been made in the first step in an experimental setup, suggest that levosimendan could be an interesting approach in treating patients after SAH for preventing dCVS. It is also possible that levosimendan has a beneficial effect on the ET-(A) receptor, resulting in a partly decreased ET-(A)-dependent-enhanced vasocontraction after SAH. To determine an ET-(A) receptor-dependent effect, more investigations are required. Another possible effect in the prevention and/or treatment of dCVS after SAH could be identified by inhibition of the PGF-induced contraction. Therefore, levosimendan shows multiple positive qualities for reducing dCVS after SAH.

Another positive effect, which has not been investigated in this study, is the inodilative effect to the heart, which results in a higher mean arterial blood pressure [22], already used for triple h-therapy. After SAH, another severe complication is the potential development of acute heart failure like takotsubo cardiomyopathy, a disease for which the use of adrenergic substances is contraindicated. In the literature there are already several studies that have shown a beneficial effect of levosimendan administration for improving outcome in patients after SAH complicated by acute heart failure [5, 30, 39].

Levosimendan seems to have beneficial effects in several parts of a multifactorial process, which is made responsible for the poor overall outcome after SAH. In addition to its neuroprotective effects [32, 33], levosimendan seems to reduce the inflammation and the early brain injury process after stroke and spinal cord injury [12, 14]. Cerebral autoregulation could be additive restored in a middle cerebral artery occlusion

model [4] and as the present data clearly emphasize, levosimendan could prevent and/or reduce dCVS after SAH.

Except for the lack of data for prevention of cortical spreading depression, levosimendan seems to affect most of the processes that determine the outcome of patients after SAH. After SAH, the functional loss of the ET-(B_1) receptor-mediated vasorelaxation is one of the factors leading to cerebral vasospasm [2]. Therefore, levosimendan seems to be an interesting therapeutic approach in preventing/antagonizing dCVS [20], however further investigations are needed.

Conclusions

To our knowledge, this is the first study that describes functional changes of the cerebrovascular contractility by levosimendan after experimental-induced dCVS in vitro.

Levosimendan treatment results in non-significantly reduced ET-1-dependent contraction. Whether this effect is ET-(A) or ET-(B_1) receptor-dependent remains to be seen. We showed that levosimendan is especially able to influence the ET-(B_1) receptor positively, which is normally functionally impaired after SAH. In addition, levosimendan reversed PGF contraction dose-dependently.

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Compliance with ethical standards

Funding No funding was received for this research.

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Animal experiments All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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