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(54) Titre : UTILISATION D'UN INHIBITEUR DE LA KALLIKREINE POUR LA PRODUCTION D'UN COMPOSE PHARMACEUTIQUE POUR LA PROPHYLAXIE ET LE TRAITEMENT DE CERTAINES MALADIES

(54) Title: USE OF KALLIKREIN INHIBITOR FOR THE PRODUCTION OF A PHARMACEUTICAL FOR THE PROPHYLAXIS AND THERAPY OF CERTAIN DISEASES

(57) Abrégé/Abstract:
The use of C1 inactivator for the production of a pharmaceutical for the prophylaxis and treatment of capillary leak syndrome (generalized extravasation) and circulatory shock (refractory hypotension) in severe burns or scalds, in polytrauma, in operations under conditions of extracorporeal circulation, in the use of cytokines, endogenous mediators, and mediator hybrids and growth factors produced by genetic engineering, or capillary leak syndrome and veno-occlusive disease of the liver in therapeutically or prophylactically indicated bone marrow transplantation is described.

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Abstract

Use of a kallikrein inhibitor for the production of a pharmaceutical for the prophylaxis and therapy of certain diseases

The use of C1-inactivator for the production of a pharmaceutical for the prophylaxis and treatment of capillary leak syndrome (generalized extravasation) and circulatory shock (refractory hypotension) in severe burns or scalds, in polytrauma, in operations under conditions of extracorporeal circulation, in the use of cytokines, endogenous mediators, and mediator hybrids and growth factors produced by genetic engineering, or capillary leak syndrome and veno-occlusive disease of the liver in therapeutically or prophylactically indicated bone marrow transplantation is described.

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Use of a kallikrein inhibitor for the production of a pharmaceutical for the prophylaxis and therapy of certain diseases

The invention relates to the use of C1-inactivator for the production of a pharmaceutical for the prophylaxis and treatment of capillary leak syndrome (generalized extravasation) and circulatory shock (refractory hypotension) in severe burns or scalds, in polytrauma, in operations under conditions of extracorporeal circulation, in the use of cytokines, endogenous mediators, and mediator hybrids and growth factors produced by genetic engineering, or capillary leak syndrome and veno-occlusive disease of the liver in therapeutically or prophylactically indicated bone marrow transplantation.

The kallikrein system consists of a number of proteases and intermediate products which, after initial activation, lead successively to the formation of vasoactive kinins (for example bradykinin). The decisive quantity is the amount of proteolytically active kallikrein.

The activation of the kallikrein system can take place as a result of direct action of the damaging mechanisms, but also indirectly by the generation of C-reactive protein in the course of an acute phase reaction, possibly with the interposition of further protease systems.

However, independently of the underlying trigger mechanisms, the further course of the pathophysiological events is dependent on the capability of the organism to regulate the formation of kallikrein and to control the proteolytic activity of the generated kallikrein to an adequate extent by means of sufficient degradation or inhibition.

The most important physiological regulator of the kallikrein system under in vivo conditions is C1-inactivator. It displays its action on the central site of the system by interaction with the activated protease kallikrein and inhibition thereof.

The activation of the kallikrein system is observed in the course of a number of diseases and also in the course of therapeutic and/or prophylactic iatrogenic interventions. These activation processes are generally associated with a consumption of the factors involved, in particular of the inhibitor.

Although as a result of the acute phase reaction an excess production of C1-inactivator is to be observed, its inhibitory capacity is not sufficient to control the activation of the kallikrein system.

The etiopathogenesis of the occurrence of shock and of generalized edema in <u>patients with severe burns</u> is as follows: the heat trauma leads to an acute phase reaction and to the (indirect and/or direct) activation of the kallikrein system. If the inhibitor potential of the body is used up, unhindered activation and uncontrolled release of toxic intermediate products (for example kallikrein) takes place and also of likewise potentially harmful end products (kinins, especially (esp. bradykinin). This is documented, inter alia, in the knowledge that in patients with heat trauma the substrate "prekallikrein" (as a measure of the formation of kallikrein and bradykinin) is decreased as a function of the severity of the heat injury.The substances mentioned have the potential to affect the vascular endothelium directly. However, as a result of activation of secondary mechanisms (for example NO [nitric oxide], cGMP [cyclic guanosine monophosphate]), they can lead to indirect damage either in the endothelial cell wall or in the

endothelium itself. The pathomorphological result of the harmful effects on the endothelium are an extravasation from the vascular system and/or the decreased response of the vascular musculature to vasoconstrictor impulses. Clinical symptoms in the patients are generalized edema and refractory hypotension (circulatory shock).

Pathogenically, the substances mentioned have the potential to affect the vascular endothelium directly. However, as a result of activation of secondary mechanisms (for example NO, cGMP), they can either lead to indirect damage in the endothelial cell wall or in the endothelium itself. The pathomorphological result of the harmful effects on the endothelium are an extravasation from the vascular system and/or the decreased response of the vascular musculature to vasoconstrictor impulses. Clinical symptoms in the patients are generalized edema and refractory hypotension (circulatory shock).

The etiopathogenesis of the occurrence of shock in patients with polytrauma is as follows: the trauma itself or the resulting damage in the body (unstable fractures, necrotic tissue) lead to an acute phase reaction and to the (indirect and/or direct) activation of the kallikrein system. If the inhibitor potential of the organism is used up, unhindered activation of this system takes place with uncontrolled release of toxic intermediate products (for example kallikrein) and also of likewise potentially harmful end products (kinins, esp. bradykinin). These substances have the potential to affect the vascular endothelium directly. However, as a result of activation of secondary mechanisms (for example NO, cGMP), they can lead to indirect damage either in the endothelial cell wall or in the endothelium itself. In diagnostic investigations in patients with polytrauma, the substrate "prekallikrein" (as a measure of the formation of kallikrein and bradykinin) is found to be decreased. The extent of the decrease correlates with the severity of the injury. The pathomorphologic result of the harmful effects on the endothelium are an extravasation from the vascular system, but especially the decreased response of the vascular musculature to vasoconstrictor impulses. The predominant clinical symptom in the patient is refractory hypotension (circulatory shock).

The etiopathogenesis of the occurrence of shock and of generalized edema in patients in the state after therapeutically or prophylactically indicated bone marrow transplantation is as follows: ablative pretreatment (chemotherapy and/or radiotherapy) by itself or else in cooperation with the subsequent bone marrow transplantation leads to an acute phase reaction and to the (indirect and/or direct) activation of the kallikrein system. If the inhibitor potential of the organism is used up, unhindered activation of this system takes place with uncontrolled release of toxic intermediate products (for example kallikrein) and also of likewise potentially

harmful end products (kinins, esp. bradykinin). These substances have the potential to affect the vascular endothelium directly. However, as a result of activation of secondary mechanisms (for example NO, cGMP) they can also lead to indirect damage in the endothelial cell wall or in the endothelium itself. The pathomorphological result of the harmful effects on the endothelium are an extravasation from the vascular system and/or the decreased response of the vascular musculature to vasoconstrictor impulses. Clinical symptoms in the patient are so-called "veno-occlusive disease", generalized edema and refractory hypotension (circulatory shock) and/or a progressive dysfunction of one or more organ systems.

From diagnostic studies in humans, it is known that the occurrence of life-threatening complications (generalized edema and/or circulatory shock) is associated with the symptom of dysregulated activation of the kallikrein system. Complication-free intervals are accompanied by a normalization of the laboratory parameters.

The etiopathogenesis of the occurrence of shock and of generalized edema in <u>patients who undergo operations under the conditions of an extracorporeal circulation (bubble or membrane oxygenators)</u> is as follows: the contact of the blood with the foreign surfaces or its interaction with the oxygen bubbles of the oxygenator lead to an acute phase reaction and to the (indirect and/or direct) activation of the kallikrein system. If the inhibitor potential of the organism is used up, unhindered activation of this system takes place with uncontrolled release of toxic intermediate products (for example kallikrein) and also of likewise potentially harmful end products (kinins, esp. bradykinin). These substances have the potential to affect the vascular endothelium directly. However, as a result of activation of secondary mechanisms (for example NO, cGMP), they can

also lead to indirect damage either in the endothelial cell wall or in the endothelium itself. The pathomorphological result of the harmful effects on the endothelium are an extravasation from the vascular system and/or the decreased response of the vascular musculature to vasoconstrictory impulses. Clinical peri- and postoperative symptoms in the patient are generalized edema and/or refractory hypotension (circulatory shock) and/or a reduction in the cardiac output.

The etiopathogenesis of the occurrence of shock and of generalized edema in patients under administration of cytokines, endogenous mediators, mediator hybrids and growth factors produced by genetic engineering in the course of the therapeutic use of these substances individually, in combination with one another or in combination with other therapeutic or prophylactic measures is as follows: the administration of these abovementioned substances, individually, in combination with one another or in combination with other therapeutic or prophylactic measures leads to an acute phase reaction and to the (indirect and/or direct) activation of the kallikrein system. If the inhibitor potential of the organism is used up, unhindered activation of this system takes place with uncontrolled release of toxic intermediate products (for example kallikrein) and also of likewise potentially harmful end products (kinins, esp. bradykinin).

The pathogenetic potential of these substances is that they are directly able to affect the vascular endothelium. However, as a result of activation of secondary mechanisms (for example NO, cGMP) they can also lead to indirect damage either in the endothelial cell wall or in the endothelium itself. In addition, however, some of the abovementioned cytokines, endogenous mediators and growth factors (for example interleukin-1β, tumor necrosis factor, and interferons) can also have a

damaging effect directly on the endothelium. Others, however, also have an indirect effect in that they induce increased production or activation of the directly damaging cytokines. In some of the cytokines, endogenous mediators and growth factors, it has still not been possible to elucidate the exact pathological mechanism of the endothelial damage. The pathomorphological result of the damaging effects on the endothelium are an extravasation from the vascular system and/or the decreased response of the vascular musculature to vasoconstrictor impulses. Clinical symptoms in the patient are generalized edema and refractory hypotension (circulatory shock.)

Accordingly, severe burns, polytrauma, therapeutic or prophylactically indicated bone marrow transplantations, operations under conditions or extracorporeal circulation and also the therapeutic or prophylactic use of cytokines, endogenous mediators, and mediator hybrids and growth factors produced by genetic engineering have, as an accompanying symptom, generalized edema and refractory hypotension (circulatory shock) in common. The symptoms mentioned are often seen after activation of the kallikrein system.

We have now found that the administration of C1-inactivator has a positive effect in the treatment of generalized edema, refractory hypotension (circulatory shock), progressive dysfunction of organ systems and reduced cardiac output (in patients under extracorporeal circulation).

The invention accordingly relates to the use of C1-inactivator for the production of a pharmaceutical for the prophylaxis and treatment of capillary leak syndrome (generalized extravasation) and circulatory shock (refractory hypotension) in severe burns or scalds, in polytrauma, in operations under conditions of extracorporeal circulation, in the use of cytokines, endogenous mediators, and mediator hybrids and growth factors produced by genetic engineering, or capillary leak syndrome and veno-occlusive disease of the liver in therapeutically or prophylactically indicated bone marrow transplantation.

C1-inactivator can be used, which can be prepared from blood plasma in a manner known to the person skilled in the art, and preferably as a purified product.

C1-inactivator is known as a pharmaceutical as a pyrogen-free lyophilisate, which is dissolved before administration and preferably injected intravenously.
One unit of the C1-inactivator concentrate corresponds to the activity of 1 ml of pooled human citrate plasma (1 unit [1 U] thus corresponds to 6 Levy & Lepow units).

C1-inactivator expressed by genetic engineering and purified can also be employed for the production of the pharmaceutical.

The pharmaceutical can be prepared for intravenous (bolus or infusion), intramuscular or subcutaneous administration.

The pharmaceutical contains 1 - 5,000 U/kg of bodyweight (BW)/day, preferably 5 - 1,000 U/kg BW/day of C1-inactivator.

For adults, a solid pharmaceutical having a dose of 1 - 300,000 U/day, preferably 50 - 60,000 U/day of C1-inactivator, can also be prepared.

C1-inactivator can be used separately or as a combination with other pharmaceutical substances. Particularly in combination with pharmaceutical auxiliaries, the production of an oral or rectal form is also possible.

Example of clinical uses of the pharmaceutical according to the invention:

In patients with generalized edema and refractory hypotension (circulatory shock) in the course of heat injuries (burning, scalding), the high-dose intravenous use of C1-inactivator concentrate led to breaking of refractory hypotension and also to improvement of generalized edema. The therapeutic administration scheme used was as follows:

initially	5,000 U of C1-inactivator i.v.,
after 12 hours	2,500 U of C1-inactivator i.v.,
after a further 12 hours	1,500 U of C1-inactivator i.v.,
after a further 12 hours	1,000 U of C1-inactivator i.v.

In patients with polytrauma, the use of C1-inactivator concentrate led to the correction of the desperate circulatory situation. The treatment schemes used differed, but varied in order of magnitude from 1,000 to 6,000 U of C1-inactivator concentrate i.v. and were as a rule administered repetitively at 12 hour intervals.

In patients with therapeutically or prophylactically indicated bone marrow transplantation, C1-inactivator concentrate was employed i.v. in two patients. The dose scheme was:

initially	60 U/kg of BW
after 12 hours:	30 U/kg of BW
after 12 hours:	30 U/kg of BW
after 12 hours:	15 U/kg of BW
after 12 hours:	15 U/kg of BW
after 12 hours:	15 U/kg of BW

In these patients, successful treatment of generalized edema, the beginnings of "veno-occlusive disease" and also (in one case) the beginnings of renal failure was possible.

In patients who have been subjected to an operation under conditions of extracorporeal circulation, C1-inactivator concentrate was administered i.v. in altogether 56 patients. In 55 patients, this was carried out in the course of a clinical trial in the indication "bypass operation", in one patient, a newborn child, this was carried out in the course of a therapeutic "off label" use during a so-called transposition operation of the aorta and pulmonary artery. The results were as follows:

In the adult patients who received C1-inactivator concentrate in the course of a bypass operation, it was possible in those who preoperatively suffered from a reduced cardiac output to distinctly improve this in the peri- and post-operative course.

In the newborn child, a generalized edema occurred in the post-operative course. It was possible to control this successfully with C1-inactivator concentrate and it resolved.

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THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. The use of C1-inactivator for the production of a pharmaceutical for the prophylaxis and treatment of capillary leak syndrome (generalized extravasation) and circulatory shock (refractory hypotension) in severe burns or scalds, in polytrauma, in operations under conditions of extracorporeal circulation, in the use of cytokines, endogenous mediators, and mediator hybrids and growth factors produced by genetic engineering, or capillary leak syndrome and veno-occlusive disease of the liver in therapeutically or prophylactically indicated bone marrow transplantation.

2. The use as claimed in claim 1, wherein a composition containing 1 to 5,000 U/kg BW/day, preferably 5 to 1,000 U/kg BW/day, of C1-inactivator is prepared.

3. The use as claimed in claim 1, wherein a solid composition containing C1-inactivator 1 to 300,000 U/BW/day, preferably 50 to 60,000 U/BW/day, is prepared.

4. The use as claimed in claim 1, wherein a composition for intravenous, intramuscular and subcutaneous use is prepared, preferably in combination with pharmaceutical auxiliaries.

5. The use as claimed in claim 4, wherein a composition for oral or rectal use is prepared.