Intensive Care Med (1996) 22:1214–1223

EXPERIMENTAL

M. Fischer
B. W. Böttiger
S. Popov-Cenic
K.-A. Hossmann

Thrombolysis using plasminogen activator and heparin reduces cerebral no-reflow after resuscitation from cardiac arrest: an experimental study in the cat

Received: 6 November 1995
Accepted: 13 June 1996

Presented in part at the 1994 Annual Meeting of the American Society of Anesthesiologists, 18 October 1994, Washington, DC, and at the 39th Annual Meeting of the German Association of Thrombosis and Hemostasis, 17 February 1995, Berlin, Germany

M. Fischer[1] · B. W. Böttiger[2]
K.-A. Hossmann (✉)
Max-Planck-Institute
for Neurological Research,
Department of Experimental Neurology,
Gleueler Strasse 50,
D-50931 Köln, Germany
FAX: +49(221)4726325

S. Popov-Cenic
Department of Experimental Hematology
and Transfusion Medicine,
University of Bonn, Germany

[1] On leave from the Department of Anesthesiology and Intensive Care Medicine, Bonn, Germany
[2] On leave from the Department of Anesthesiology, University of Heidelberg, Germany

Abstract *Objective:* Successful resuscitation of the brain requires complete microcirculatory reperfusion, which, however, may be impaired by activation of blood coagulation after cardiac arrest. The study addresses the question of whether postischemic thrombolysis is effective in reducing cerebral no-reflow phenomenon.
Design: 14 adult normothermic cats were submitted to 15-min cardiac arrest, followed by cardiopulmonary resuscitation (CPR) and 30 min of spontaneous recirculation. The CPR protocol included closed-chest cardiac massage, administration of epinephrine 0.2 mg/kg, bicarbonate 2 mEq/kg per 30 min, and electrical defibrillation shocks.
Interventions: During CPR, animals in the treatment group ($n = 6$) received intravenous bolus injections of 100 U/kg heparin and 1 mg/kg recombinant tissue type plasminogen activator (rt-PA), followed by an infusion of rt-PA 1 mg/kg per 30 min.
Measurements and results: Microcirculatory reperfusion of the brain was visualized by labeling the circulating blood with 300 mg/kg of 15% fluorescein isothiocyanate albumin at the end of the recirculation period. Areas of cerebral no-reflow – defined as the absence of microvascular filling – were identified by fluorescence microscopy at eight standard coronal levels of forebrain, and expressed as the percentage of total sectional area. One animal in the treatment group was excluded from further analysis because of intracerebral hemorrhage due to brain injury during trepanation. Autopsy revealed the absence of intracranial, intrathoracic, or intra-abdominal bleeding in all the other animals. In untreated animals ($n = 8$), no-reflow affected 28±13% of total forebrain sectional areas, and only 1 out of 8 animals showed homogenous reperfusion (i.e., no-reflow <15% of total forebrain sectional areas). Thrombolytic therapy ($n = 5$) significantly reduced no-reflow to 7±5% of total forebrain sectional areas and all treated animals showed homogeneous reperfusion at the microcirculatory level.
Conclusions: The present data demonstrate that thrombolytic therapy improves microcirculatory reperfusion of the cat brain when administered during reperfusion after cardiac arrest.

Key words Brain resuscitation · Cardiac arrest · Cerebral ischemia · Microcirculation · Thrombolytic therapy

Introduction

Injury to the brain following a period of circulatory arrest depends not only on the duration of ischemia but also on various postischemic events which aggravate the primary lesion. Two forms of postischemic disturbances can be differentiated: hemodynamic disturbances which interfere with homogeneous reoxygenation of the brain, and metabolic disturbances which may cause delayed neuronal death in vulnerable brain regions [1]. In animals, delayed neuronal death is probably of little significance, because neurological recovery is only slightly impaired if the ischemic lesion is restricted to selectively vulnerable brain regions [2–4]. However, there is strong evidence that hemodynamic disturbances, such as the no-reflow phenomenon [5–8] and the postischemic hypoperfusion syndrome [1, 8], are of considerable importance, because these disturbances result in damage to cortical neurons, which are mainly responsible for the poor neurological outcome [9, 10].

We recently observed that resuscitation of the heart via closed-chest massage and intravenous administration of epinephrine after cardiac arrest caused severe no-reflow of the brain [11]. This disturbance is reversible when cardiopulmonary resuscitation (CPR) starts within 5 min after initiation of circulatory arrest, but it persists when cardiac arrest is prolonged [11]. Factors that modulate the severity of no-reflow are the duration of ischemia [6, 7, 11], reperfusion pressure [7, 12], swelling of endothelial cells [13], and hemoconcentration [14]. There are also indications that the activation of the coagulation cascade after ischemia promotes the development of no-reflow [15–17]. In fact, it was demonstrated that fibrinogen levels decrease [16, 18] and plasma clotting times increase [16] following cerebral ischemia induced by either arterial inflow occlusion or cardiac arrest. This activation may lead to the entrapment of platelets within the cerebral microcirculation [17] and may cause the formation of microthrombi in the pial vessels of the cortex [15].

The pathophysiological importance of coagulation disturbances is supported by the observation that the quality of electrophysiological recovery is inversely related to the severity of postischemic coagulopathy [16]. Efforts have, therefore, been made to prevent coagulopathy by heparin treatment. In fact, beneficial effects of heparin treatment were found if the drug was either applied before the induction of ischemia [19] or the duration of ischemia was restricted to a maximum of 7 min [20]. Amelioration of ischemic damage by heparin, however, could not be detected when ischemia was prolonged to 15 min [21]. Obviously, the failure of heparin to improve brain injury after prolonged ischemia is due to the fact that this drug is not able to disaggregate formed thrombi. We, therefore, supplemented heparin with recombinant tissue-type plasminogen activator (rt-PA) to improve cerebral reperfusion after prolonged cardiac arrest and resuscitation.

Materials and methods

Animal preparations

The experiments were carried out according to the Society for Neuroscience guidelines for the use of animals in neuroscience and were approved by the ethical committee of the local authorities.

After overnight fasting and free access to water, 14 mongrel cats (2.5–4.6 kg) were premedicated with an intramuscular injection of ketamine 10 mg/kg. The animals were anesthetized using 1.0% halothane in 70% nitrous oxide and 30% oxygen via a face mask. The trachea was intubated and the ventilator was adjusted to achieve an arterial partial pressure of carbon dioxide of 30 mmHg, which reflects the normal value in cats. A 5 F triple-lumen catheter was inserted into the left femoral vein for continuous monitoring of central venous pressure and for drug administration. Both femoral arteries were cannulated to measure aortic and left ventricular pressures. The left ventricle was cannulated retrograde using a 3 F single-lumen pigtail catheter. With the insertion of the venous line, an infusion of Ringer solution (4 ml/kg per h) was started and maintained during the entire study period before and after cardiac arrest. Heparin administration during the preparation and control periods was strictly avoided in all animals; arterial lines were flushed with Ringer solution. The animals were kept at a rectal temperature of 37 °C using a feedback-controlled heating system.

The animal's head was mounted in a stereotactic frame. Two cranial windows of 5-mm diameter were prepared on the left side over the frontal and the parietal region using a dental drill. The dura remained intact over both windows. Two plexiglas cylinders were attached watertight to the skull with dental cement. The frontal cylinder was connected to a pressure transducer (Kombidyn, B. Braun Medical, Melsungen, Germany) by a catheter filled with lactated Ringer solution for measurement of intracranial pressure. A laser-Doppler-flow probe (Pf2b, Perimed, Stockholm, Sweden) was inserted into the parietal cylinder for continuous monitoring of cerebral blood flow (CBF). Two frontoparietal screw electrodes were used to monitor the electroencephalogram (EEG). The electrocardiogram (ECG) was recorded from bipolar esophageal electrode using differential amplifiers (Low Level Amplifier 122, Tektronic, Portland, CA, USA).

To avoid potentially adverse side effects of halothane on cardiac function, the halothane concentration was terminated after surgery in a stepwise fashion and replaced by infusions of alfentanil (0.3 mg/kg per h) and midazolam (0.75 mg/kg per h). EEG, ECG, and CBF, aortal, left ventricular, central venous, and intracranial pressures were recorded continuously or an eight-channel polygraph (5525C Dynograph, Beckman Instruments, IL, USA). EEG analysis and data acquisition were carried out using a Macintosh II fx computer (Apple, Cupertino, CA, USA), an analog/digital converter (Macadios, GW Instruments, Sommerville, MA, USA), and the appropriate software (Superscope, Macadios, GW Instruments, Sommerville, MA, USA). Traces of 30-s duration were recorded with a sampling rate of 125 Hz, processed, and written to a database. For each trace period, the EEG amplitude, the median frequency of the power spectrum, and the relative power of the alpha, beta, theta, and delta frequency bands were determined. Every 30-s arterial, central venous, and intracranial pressures were averaged. Left ventricular and aortic diastolic pressures were defined as the minimum values of each trace. Cerebral perfusion pressure was calculated from the difference of the mean arterial blood pressure and the higher value of either the intracranial or the central venous pressure. Myocardial perfusion pressure was calculated from the difference between diastolic aortic and diastolic left ventricular pressure. To determine left ventricular inotropy, left ventricular pressure was differentiated and the maximum of dp/dt was calculated.

Experimental protocol

After a control period of 30 min, cardiac arrest was induced at 37 °C by internal electrical stimulation via a bipolar esophageal electrode (50 Hz, 5 mA, 70 V). At the same time, ventilation and infusions were stopped and the heating system was switched off. After 15 min of ventricular fibrillation, CPR was started using closed-chest cardiac massage at a compression rate of about 120/min. Ventilation with 100% oxygen was resumed at a ventilation rate of 140% of control. Epinephrine 0.2 mg/kg was injected and an infusion of bicarbonate 2 mEq/kg per 30 min was started. Four minutes later, the first defibrillation (Melacard Econ, Mela, München, Germany) was attempted to restore sinus rhythm. If the countershock failed, additional epinephrine 0.1 mg/kg was injected, followed by 2 min of closed-chest cardiac massage and the next defibrillation attempt. After restoration of spontaneous circulation, a 30-min period of recirculation was allowed. If mean arterial blood pressure decreased below 80 mmHg, dopamine (5–10 µg/kg per min) and epinephrine (0.05–0.2 µg/kg per min) were infused, as required.

Experimental groups

Cerebral microcirculatory reperfusion was investigated in two groups of animals. Group 1 (untreated animals, $n = 8$) received standard resuscitation procedures as described above. Group 2 (treated animals, $n = 6$) additionally received an intravenous bolus injection of rt-PA 1 mg/kg (Thomae, Biberach, Germany) and heparin 100 U/kg immediately after initiation of closed-chest cardiac massage, which was followed by a continuous infusion of rt-PA 1 mg/kg per 30 min [22].

Measurement of cerebral no-reflow

The severity of no-reflow was assessed 30 min after the return of spontaneous recirculation in both groups. The circulating blood was labeled by an intravenous bolus injection of 300 mg/kg fluorescein isothiocyanate (FITC) albumin in 2 ml/kg Ringer solution, as described elsewhere [11]. The technique of FITC-dextran injection is efficient for staining all existing capillaries in rat brain within 5 s [23]. In our study, however, we allowed dye circulation for 2 min to guarantee staining of all perfused capillaries, even under conditions of severely reduced flow rates [7]. Then the animals were killed by intravenous injection of 1 mmol/kg KCl. The brains were immediately removed, fixed in 4% formalin, and cut into standard coronal sections of 200 µm thickness [5, 7, 11]. Eight standard coronal levels of forebrain at Horsley-Clark coordinates A26–P2, with an interslice gap of approximately 4 mm, were investigated. No-reflow was defined as complete absence of capillary dye filling, whereas all other regions, even those showing abnormal staining of capillaries, were defined as reperfused tissue. Using these definitions, the whole slices were scanned by fluorescence microscopy (Axiovert 10, Zeiss, Oberkochem, Germany) at magnifications of 50- to 200-fold to identify areas of no-reflow. To avoid fading artifacts, the outlines of no-reflow were marked on light microscopy photographs of the same sections, the corresponding areas were measured using the processing software Image (National Institutes of Health, Bethesda, MD, USA), and the results were expressed as a percentage of the total sectional area. In sham-operated control cats previously investigated by the same method, all brain regions were completely perfused [11].

Laboratory analysis

Samples of arterial blood were withdrawn for assessment of blood gases, pH, serum sodium, serum potassium, serum osmolality, hematocrit, plasma glucose, and plasma lactate concentrations at 15-min prearrest, and after 5, 15, and 30 min of spontaneous recirculation. Blood gases, potassium, and sodium were measured using the Combi Analysator MT55 (Eschweiler, Kiel, Germany), glucose using the Glucose Analyzer 2 (Beckman Clinical Inst., Fullerton, CA, USA), lactate using Model 27 (Yellow Springs Instruments, Yellow Springs, OH, USA), and osmolality using the Vapour Pressure Osmometer (Wescor, Logan, OH, USA). At the same time points, a detailed analysis of the coagulation system was performed in all treated animals. For this purpose, an additional 2 ml of blood was withdrawn into a syringe containing 0.2 ml of 3.8% sodium citrate. The probe was centrifuged at 4700 rpm for 30 min. Three aliquotes of plasma were prepared and stored at −80 °C until analysis. Levels of α_2-antiplasmin (Kabi, Essen, Germany), fibrinogen (Baxter, Unterschleißheim, Germany), thrombin-antithrombin III complex (TAT) (Behring, Marburg, Germany), antithrombin III (IL, Kirchheim, Germany) were measured to detect hemostatic changes. These coagulation data were compared to those obtained from six additional untreated cats subjected to 15-min cardiac arrest and the same resuscitation protocol. Before animal experiments were started, we performed qualitative in vitro experiments which demonstrated a thrombolytic activity of human rt-PA in cat blood. At rt-PA concentrations above 10 µg/ml, clots began to disaggregate. Consistent with our findings, Korninger and Collen [24] observed a 60% activity of human rt-PA in cat plasma.

Statistical analysis

All data are expressed as means ± SD. Differences between groups or time points were analyzed for significance by the χ^2-test, Mann-Whitney U test, Student's t-test, or ANOVA, followed by Fisher's PLSD test for multiple comparisons. Statistical significance was assumed for $p < 0.05$.

Results

One animal in the rt-PA-treated group (group 2) was excluded from data analysis because of intracerebral hemorrhage due to iatrogenic brain injury during prearrest trepanation. In all other animals, autopsy did not detect any intracranial, intrathoracic, or intra-abdominal bleeding.

Hemodynamic variables

During prearrest steady-state, the hemodynamic variables of animals in both the untreated (group 1) and the rt-PA-treated (group 2) animals were in the physiological range. Differences between the groups were not be observed, except for a slightly lower myocardial perfusion pressure in group 2 (Table 1). In all animals, ventricular fibrillation was achieved within 45 s and promptly caused circulatory arrest (Fig. 1). During closed-chest cardiac

Table 1 Hemodynamic variables before cardiac arrest, during cardiopulmonary resuscitation, and after restoration of spontaneous circulation *ROSC*. Values are means ± SD (*MAP* mean arterial blood pressure, *CVP* central venous pressure, *ICP* intracranial pressure, *CPP* cerebral perfusion pressure, *CBF* cerebral blood flow measured with laser-Doppler, *LVDP* left ventricular diastolic pressure, *MPP* myocardial perfusion pressure, *CPR* cardiopulmonary resuscitation, *ND* not determined)

Experimental group	Time	MAP (mmHg)	CVP (mmHg)	ICP (mmHg)	CPP (mmHg)	CBF (% of control)	LVDP (mmHg)	MPP (mmHg)	dt/dt max (mmHg/s)
Untreated ($n = 8$)	Prearrest	107 ± 26	4 ± 3	4 ± 2	101 ± 26	100	7 ± 6	85 ± 27	3150 ± 903
	2-min CPR	77 ± 20**	40 ± 10**	17 ± 7**	37 ± 20**	50 ± 34**	10 ± 8	37 ± 9**	ND
	4-min CPR	65 ± 18**	34 ± 12**	15 ± 6**	31 ± 20**	39 ± 27**	9 ± 7	37 ± 12**	ND
	4 min after ROSC	164 ± 23**	8 ± 6**	16 ± 8**	147 ± 17**	141 ± 61	14 ± 11**	120 ± 18**	3994 ± 1013**
	14 min after ROSC	126 ± 33	8 ± 6**	9 ± 4**	116 ± 28	149 ± 74	7 ± 8	97 ± 27	3465 ± 1128
	30 min after ROSC	92 ± 19	7 ± 4	5 ± 3	85 ± 16	144 ± 57	3 ± 4	68 ± 11	3267 ± 699
rt-PA lysis ($n = 5$)	Prearrest	100 ± 31	5 ± 2	8 ± 2	93 ± 32	100	3 ± 3	62 ± 10*	2688 ± 477
	2-min CPR	64 ± 12**	31 ± 19**	16 ± 4**	32 ± 13**	67 ± 38**	7 ± 4**	32 ± 9**	ND
	4-min CPR	57 ± 9**	26 ± 11**	15 ± 3***	30 ± 15**	62 ± 32**	9 ± 6**	24 ± 3***	ND
	4 min after ROSC	138 ± 25	10 ± 3**	22 ± 6**	117 ± 25*	173 ± 72	9 ± 10	102 ± 32**	4575 ± 1187**
	14 min after ROSC	135 ± 19	9 ± 3**	16 ± 3*	119 ± 20	174 ± 65	2 ± 3	108 ± 11**	4743 ± 238***
	30 min after ROSC	88 ± 21	7 ± 3	5 ± 4	81 ± 18	149 ± 46	0 ± 1*	68 ± 19	3897 ± 400

* Significantly different from untreated animals ($p < 0.05$, Student's t-test); ** significantly different from prearrest control values ($p < 0.05$, ANOVA)

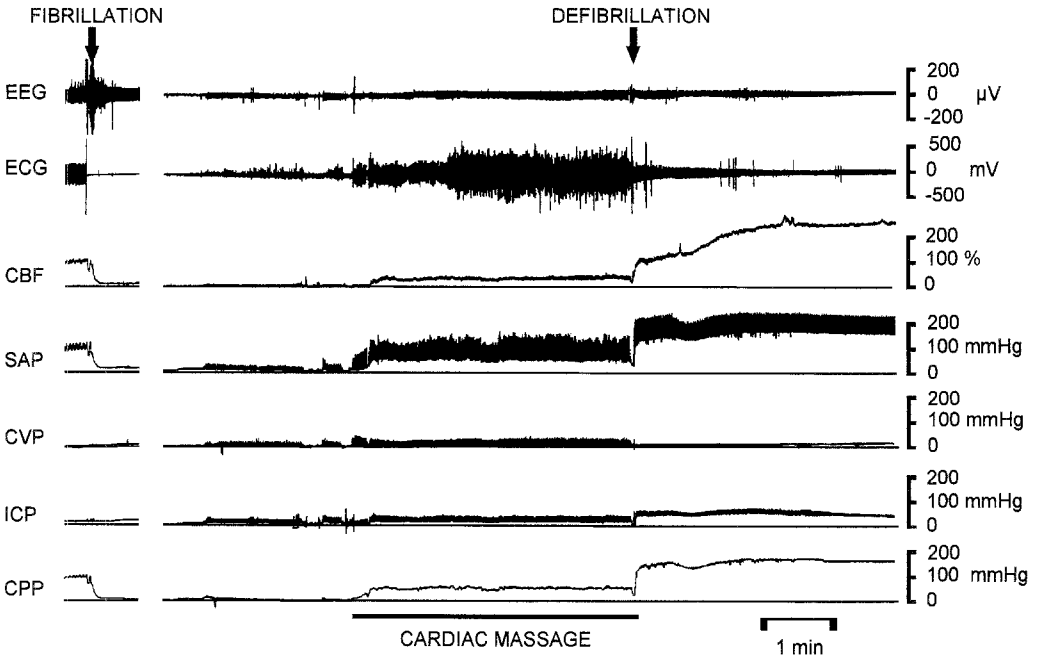


Fig. 1 Cardiopulmonary resuscitation after 15-min cardiac arrest in a cat treated with a bolus injection of rt-PA 1 mg/kg and heparin 100 U/kg, followed by infusion of rt-PA 1 mg/kg per 30 min. A polygraphic recording of the onset of cardiac arrest, closed-chest cardiac massage, defibrillation, and early reperfusion is shown. High central venous *CVP* and intracranial pressure *ICP* during closed-chest cardiac massage resulted in low cerebral blood flow *CBF*, measured by laser-Doppler, which, however, rapidly recovered to above prearrest levels after restoration of spontaneous circulation. *EEG* electroencephalogram, *ECG* electrocardiogram, *SAP* systemic arterial pressure

massage the hemodynamic variables were comparable in both groups. CBF reached 30 to 60% of prearrest values, and perfusion pressures of heart and brain were reduced to approximately 35% of prearrest values (Table 1). Restoration of spontaneous circulation was achieved in all animals within 6 min.

Following successful defibrillation, a hypertensive episode of about 15-min duration was observed in all cats. Arterial blood pressure then returned to normal range, and was stabilized near to prearrest levels using low infusion rates of dopamine and epinephrine. During

recirculation, treated cats exhibited a significantly higher intracranial pressure which, in combination with a slightly lower arterial pressure, resulted in a significantly lower cerebral perfusion pressure. Laser-Doppler-flow, however, tended to be higher in the cerebral cortex of rt-PA-treated animals (Table 1, Fig. 2). Moreover, cardiac inotropy determined by the calculation of dp/dt max reached significantly higher levels in treated cats, which suggested an improvement in cardiac function due to the treatment strategy (Table 1, Fig. 2).

Blood variables

During the prearrest control period, laboratory parameters in both groups were found to be within the normal range (Table 2).

Cardiac arrest and CPR led to severe metabolic disturbances, as evidenced by blood lactic acidosis and an increase in serum glucose. Arterial pH remained at about 7.15 until the end of the recirculation period. Serum lactate and glucose slowly decreased during reperfusion but did not reach baseline levels. Treatment with rt-PA, however, accelerated this decline significantly. Cardiac arrest and resuscitation caused hemoconcentration despite continuous infusion of Ringer solution 4 ml/kg per h before and after cardiac arrest. The hematocrit increased in both groups after resuscitation and did not normalize within 30 min of recirculation. In both groups, ventilation with pure oxygen caused an increase in the partial pressure of oxygen in arterial blood to about 300 mmHg after resuscitation, indicating that pulmonary function was not severely disturbed by cardiac arrest and CPR. Increasing the ventilation rate to 140% of control prevented hypercapnia caused by bicarbonate administration in treated but not in untreated animals.

The effects of cardiocirculatory arrest with and without thrombolytic therapy on the coagulation system are shown in Fig. 3. In untreated animals, the activation of the coagulation cascade after CPR is indicated by an increase in TAT levels and a decrease in fibrinogen concentrations. Treatment with rt-PA and heparin diminished the increase in TAT levels after CPR. Fibrinogen concentrations, however, decreased to a significantly lower level, indicating an unspecific fibrinogenolytic activity of rt-PA. The specificity of the in vivo effects of rt-PA and heparin in cats is reflected by decreasing concentrations of α_2-antiplasmin.

Electrophysiological observations

During the prearrest control period, the animals exhibited the characteristic EEG pattern of alfentanil and midazolam anesthesia. The median EEG frequency was between 4 and 6 Hz and the main EEG activity was in the frequency range from 0.5 to 7 Hz. Following induction of ventricular fibrillation, the EEG flattened within 20 s in all animals. After cardiac arrest and CPR, EEG began to recover but did not normalize within the survival period of 30 min. In both groups, EEG power returned to only 15% of prearrest control. The pupils completely dilated in all animals within 1 min after ventricular fibrillation. Fifteen minutes after successful resuscitation, the pupils began to constrict and two animals in each group demonstrated nearly complete miosis at the end of the experiment.

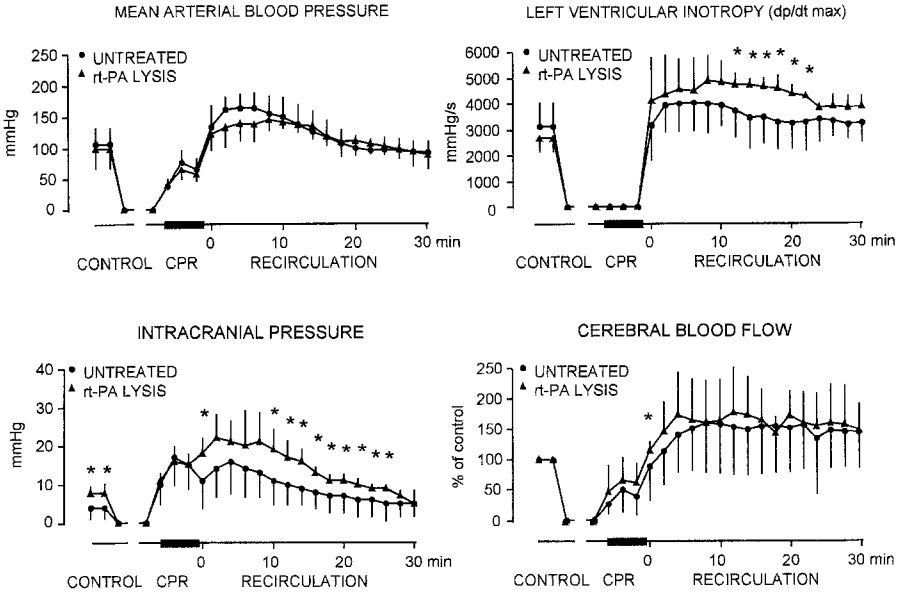


Fig. 2 Mean arterial blood pressure, left ventricular inotropy, intracranial pressure, and cerebral blood flow (measured by laser-Doppler in the parietal cortex) during control period, closed-chest cardiac massage (CPR), and after resuscitation (recirculation). Note the absence of major differences between untreated and treated animals in cerebral blood flow, despite increased intracranial pressure in treated cats (treatment: bolus injection of rt-PA 1 mg/kg and heparin 100 U/kg, followed by infusion of rt-PA 1 mg/kg per 30 min). Values are means ± SD. * Significant difference between treated and untreated animals ($p < 0.05$, Student's t-test)

Table 2 Blood parameters before cardiac arrest and after cardiopulmonary resuscitation. Values are means ± SD (PaO_2 partial pressure of oxygen and $PaCO_2$ partial pressure of carbon dioxide in arterial blood, *Hct* hematocrit, *ROSC* restoration of spontaneous circulation)

Experimental group	Time	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH (units)	Lactate (mmol/l)	Hct (%)	Serum Na (mmol/l)	Serum K (mmol/l)	Glucose (mol/l)	Osmolality (mOsm/l)
Untreated ($n = 8$)	Prearrest	161 ± 21	30 ± 2	7.34 ± 0.08	1.2 ± 0.4	39 ± 3	144 ± 8	4.1 ± 0.9	7.5 ± 1.3	299 ± 8
	5 min after ROSC	287 ± 95**	38 ± 9**	7.10 ± 0.16**	5.3 ± 0.6**	44 ± 4**	148 ± 12	4.3 ± 1.3	13.5 ± 1.0**	312 ± 7**
	15 min after ROSC	344 ± 115**	42 ± 9**	7.12 ± 0.11**	4.5 ± 0.5**	43 ± 5**	150 ± 11	3.9 ± 0.9	12.7 ± 1.3**	317 ± 10
	30 min after ROSC	336 ± 141**	40 ± 9**	7.14 ± 0.07**	4.3 ± 1.1**	43 ± 4**	145 ± 8	4.1 ± 0.7	12.6 ± 1.9**	308 ± 8**
rt-PA lysis ($n = 5$)	Prearrest	154 ± 15	30 ± 3	7.35 ± 0.10	0.9 ± 0.2	40 ± 2	135 ± 7*	3.6 ± 0.7	6.3 ± 0.8	290 ± 11
	5 min after ROSC	280 ± 146	31 ± 7	7.39 ± 0.23	4.8 ± 0.6**	43 ± 2**	144 ± 3**	4.8 ± 1.1	11.1 ± 1.7***	307 ± 3**
	15 min after ROSC	285 ± 116	34 ± 7	7.25 ± 0.18**	3.5 ± 0.5***	44 ± 4	143 ± 5	4.0 ± 0.3	10.0 ± 1.4**	311 ± 3**
	30 min after ROSC	333 ± 162**	31 ± 2	7.16 ± 0.12**	2.7 ± 0.6***	45 ± 2**	143 ± 3	3.6 ± 0.6	9.4 ± 1.2***	310 ± 7**

*Significantly different from untreated animals ($p < 0.05$, Student's *t*-test), **significantly different from prearrest control values ($p < 0.05$, ANOVA)

Severity of no-reflow

In all untreated cats, cardiac arrest of 15-min duration followed by 30-min spontaneous recirculation caused severe disturbances of microcirculation, as indicated by no-reflow in 28 ± 13% of total forebrain sectional areas (Fig. 4). Postarrest combined treatment, using rt-PA and heparin, led to a significant reduction in cerebral no-reflow to 7 ± 6% of total forebrain sectional areas (Fig. 4). Regional evaluation of no-reflow revealed a significant reduction by this treatment in all parts of the brain: in cortex from 27 ± 14% to 8 ± 6%, in basal ganglia from 63 ± 26% to 15 ± 20%, and in brainstem from 26 ± 26% to 2 ± 3% (Figs. 4, 5). All the treated animals but only one of eight untreated animals showed nearly complete reperfusion of the brain (i.e., no-reflow < 15% of total forebrain sectional areas) (χ^2-test, $p < 0.01$).

Discussion

This study clearly confirms our previous observation that cerebral microcirculatory reperfusion is severely disturbed soon after resuscitation from cardiac arrest in cats [11]. A postarrest treatment strategy, including thrombolytic therapy with rt-PA and heparin, significantly reduced the severity of cerebral no-reflow. In contrast to clinical observations which led to the contraindication of thrombolysis after resuscitation from cardiac arrest, adverse side effects were not seen, except in one animal, where the brain was iatrogenically injured during surgical preparation before administration of rt-PA.

The beneficial effect of thrombolysis using rt-PA points to fibrin formation and microthrombosis in the pathogenesis of microcirculatory impairment after prolonged cardiac arrest. In untreated cats, we observed activation of the coagulation system, shown by the increase in TAT and the decrease in fibrinogen levels. The delayed and only slight decrease in α_2-antiplasmin in the untreated animals at the end of the 30-min recirculation period confirmed the moderate activation of endogenous fibrinolysis. These data are consistent with previous experimental findings. Gaszynski [18] reported that platelet counts and fibrinogen levels decreased and plasma thrombin times shortened, indicating the development of disseminated intravascular clotting after cardiac arrest in rabbits. Standard CPR measures, including administration of epinephrine and bicarbonate, did not reverse this activation of the clotting system [18]. These authors also observed signs of a less endogenous fibrinolytic activity [18]. Hekmatpanah demonstrated that increased clotting activity caused the formation of microthrombi in cortical pial vessels within 5 to 10 min after the onset of cardiac arrest [15]. This may be a strong indication that the cerebral no-reflow phenomenon is caused, at least in part,

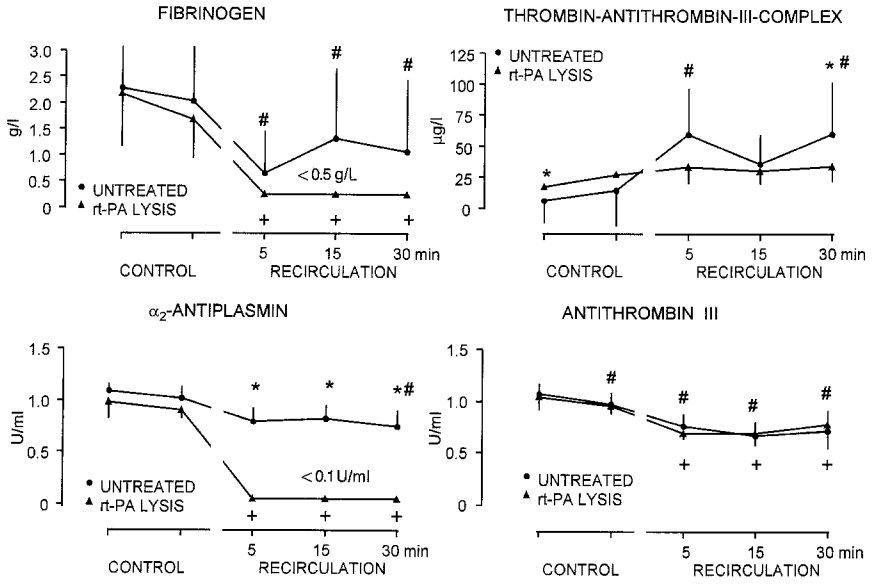


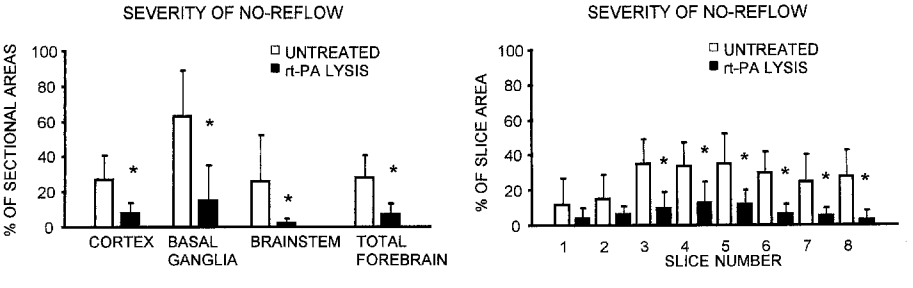
Fig. 3 Fibrinogen, thrombin-antithrombin III complex (TAT), α_2-antiplasmin, and antithrombin III during the control period and after resuscitation (recirculation). Note the activation of the coagulation cascade after CPR in untreated animals ($n = 6$), as indicated by decreased fibrinogen concentrations and increased TAT levels. Specific effects of thrombolytic therapy (bolus injection of rt-PA 1 mg/kg and heparin 100 U/kg, followed by infusion of rt-PA 1 mg/kg per 30 min) are reflected by decreasing levels of α_2-antiplasmin and fibrinogen ($n = 5$). Values are means ± SD. *Significant difference between treated and untreated animals ($p < 0.05$, Student's t-test), # significant difference from control value within untreated animals ($p < 0.05$, ANOVA), + significant difference from control value within treated animals ($p < 0.05$, ANOVA)

by activation of the coagulation system with subsequent microthrombosis.

Recently, it has been demonstrated that no-reflow increases with prolonged cardiac arrest and may affect up to 70% of the total forebrain sectional areas if CPR is started 30 min after induction of ventricular fibrillation [11]. After cardiac arrest of 15 min or longer, these microcirculatory disturbances persist for at least 30 min of recirculation, which supports the hypothesis that no-reflow is one of the main factors limiting postischemic neurological recovery [1, 11].

Several therapeutic measures have been proposed to overcome no-reflow and to ameliorate secondary ischemic lesions of the brain. Extracorporeal circulation provides normal reperfusion pressures during CPR [25–27], prevents no-reflow [25, 26], and improves cerebral recovery [27]. Small volume resuscitation [28], which is effective in normalizing circulating blood volume and nutritional blood flow after resuscitation from hemorrhagic shock [29], has also been shown to reduce cerebral no-reflow after cardiac arrest [30] and to hasten the return of neurological function after cerebral ischemia [31]. Although the pathogenetic role of leukocyte adhesion caused by increased expression of intercellular adhesion molecules during impaired reperfusion of the brain has been disputed [32], an increasing number of experi-

Fig. 4 Severity of no-reflow in various brain regions, in total forebrain, and in eight standard coronal levels of brain after 15-min cardiac arrest. Values are expressed as percentage of sectional and slice areas (means ± SD). Thrombolytic therapy (bolus injection of rt-PA 1 mg/kg and heparin 100 U/kg, followed by infusion of rt-PA 1 mg/kg per 30 min) caused a reduction in no-reflow in all brain regions, which reached statistical significance as indicated. *Significant difference between treated and untreated animals ($p < 0.05$, Mann-Whitney U test)



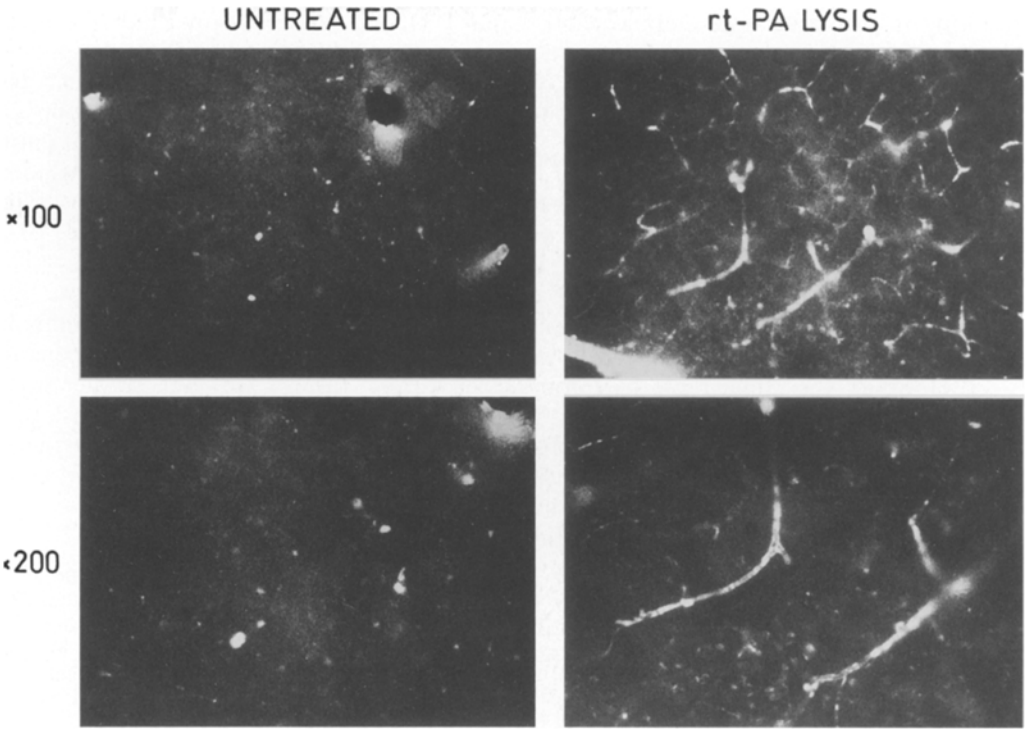


Fig. 5 Resuscitation from 15-min cardiac arrest: severely disturbed microvascular reperfusion in the caudate nucleus of an untreated animal *left* compared to homogeneous capillary filling in a cat after rt-PA and heparin treatment *right*. Fluorescence microscopy, magnification ×100 and ×200

mental studies on global or focal cerebral ischemia point to the beneficial effect of interventions that prevent or counteract the expression of adhesion molecules [33, 34]. However, to the best of our knowledge such data are not yet available for brain resuscitation after cardiac arrest.

The data from this experimental study support the hypothesis that therapeutic interventions which prevent activation of the coagulation system or increase in fibrinolytic activity improve microcirculatory reperfusion of the brain. Consistent with the findings in this study, Crowell et al. and Crowell and Smith demonstrated that pretreatment with heparin or streptokinase before cardiac arrest dramatically improved the resuscitation success and neurological recovery in dogs [35, 36]. The protease inhibitor aprotinin, in combination with heparin, corrected, in part, the activation of the clotting system and improved short-term survival after CPR in rabbits [37, 38]. Heparin treatment after cerebral ischemia of up to 7 min accelerated electrophysiological normalization and improved neurological recovery [20]. However, when ischemia was prolonged, a beneficial effect of postischemic heparin administration on cerebral resuscitation was not observed [21]. This may be due to the fact that anticoagulant treatment with heparin may prevent but does not reverse fibrin formation and microthrombotic aggregations. In contrast, rt-PA, like other thrombolytic agents, is able to dissolve even macroscopic thrombi, and therefore, is better suited to improve cerebral reperfusion under conditions of increased fibrin formation and microthombosis. In fact, Lin et al. [39] investigated the influence of streptokinase on cerebral reperfusion in a model of prolonged cardiac arrest. They demonstrated that a combination of streptokinase and dextran, but not dextran alone, ameliorated the delayed postischemic hypoperfusion syndrome and accelerated EEG recovery.

Substantial evidence has been provided that functional recovery of the brain depends on complete reperfusion [19, 27, 31, 39, 40]. In the present study, neurological recovery could not be determined, because experiments were terminated shortly after the beginning of recirculation. However, we found that in treated animals intracranial pressure was significantly elevated during the early reperfusion phase. Elevated intracranial pressure may cause a decrease in blood flow due to brain swelling, but it may also be an indicator of increased cerebral blood flow and intracranial blood volume. The latter is supported by the findings that rt-PA increased laser-Doppler flow and reduced no-reflow. If elevated intracranial pressure levels are caused by an increase in cerebral blood flow, it may correlate with improved neurological recovery, as demonstrated after experimental cerebral ischemia of 1-h duration [41].

The surprising finding of increased laser-Doppler flow despite presence of no-reflow phenomenon could be explained by microcirculatory heterogeneities. No-reflow was mainly located in the depths of the sulci and basal ganglia, whereas the superficial regions of the cortex, in which laser-Doppler flow was measured, were less affected (Fig. 4) [11]. Also, some patent vessels in the cerebral cortex may be perfused at an increased flow rate,

resulting in increased laser-Doppler flow signal [11]. A similar dissociation has been observed after massive microembolization where the vascular obstruction was also compensated by an increased flow rate through non-occluded vessels [42].

The possible clinical relevance of our findings is supported by recent observations of Böttiger et al., who demonstrated marked activation of blood coagulation after cardiac arrest in humans which was not adequately balanced by endogenous fibrinolysis [43]. If fibrin formation and microthrombosis occur after cardiac arrest, thrombolytic therapy may improve microvascular reperfusion. However, the clinical use of thrombolysis during CPR is still contraindicated, although there is growing clinical experience with thrombolysis during and after CPR in patients suffering from fulminant pulmonary embolism and myocardial infarction [44–47]. Remarkably, severe bleeding complications which could be directly attributed to CPR procedures were rarely observed, even after prolonged heart massage [44, 45]. Böttiger et al. reviewed all published material on a total of 48 patients who received thrombolysis during CPR for the treatment of massive pulmonary embolism and concluded that severe bleeding complications are mostly related to previous surgical interventions and less to CPR procedures [46]. However, under such conditions, lethal intracranial bleeding complications, a typical but rare complication of thrombolysis, may occur [46]. Weston and Avery described a higher risk of significant bleeding complications in a small group of patients who received thrombolysis after CPR and concluded that thrombolytic therapy should be initiated after CPR only when there is overwhelming ECG evidence of acute myocardial infarction [47].

In conclusion, the data from the present study support the hypothesis that thrombolytic therapy in combination with heparin improves cerebral recirculation after cardiac arrest. However, further studies are required to find out whether the potential risk of bleeding, which is related to this treatment, is outweighed by improved neurological recovery after long-term survival.

Acknowledgements The authors wish to thank Mr. B. Radermacher and Mrs. M. Jagodnik for competent technical help. The art work by Mrs. I. Mühlhöver and Mr. B. Huth and the excellent secretarial help of Mrs. M. Hahmann and Mrs. D. Schewetzky are gratefully acknowledged. Finally, we would like to acknowledge the expert laboratory work of I. Gerhard, I. Hoffmann, and E. Odenthal from the Department of Experimental Hematology and Transfusion Medicine, Bonn.

References

1. Hossmann K-A (1993) Ischemia-mediated neuronal injury. Resuscitation 26:225–235
2. Volpe BT, Pulsinelli WA, Tribuna J, Davis HP (1984) Behavioral performance of rats following transient forebrain ischemia. Stroke 15:558–562
3. Davis HP, Tribuna J, Pulsinelli WA, Volpe BT (1986) Reference and working memory of rats following hippocampal damage induced by transient forebrain ischemia. Physiol Behav 37:387–392
4. Hossmann K-A, Schmidt-Kastner P, Grosse Ophoff B (1987) Recovery of integrative central nervous function after one hour global cerebro-circulatory arrest in normothermic cat. J Neurol Sci 77:305–320
5. Ames A, Wright RL, Kowada M, Thurston JM, Majno G (1968) Cerebral ischemia. II. The no-reflow phenomenon. Am J Pathol 52:437–453
6. Ginsberg MD, Myers RE (1972) The topography of impaired microvascular reperfusion in the primate brain following total circulatory arrest. Neurology 22:998–1011
7. Fischer EG, Ames A, Lorenzo AV (1979) Cerebral blood flow immediately following brief circulatory stasis. Stroke 10:423–427
8. Hossmann K-A (1983) Neuronal survival and revival during and after cerebral ischemia. Am J Emerg Med 1:191–197
9. Horn M, Schlote W (1992) Delayed neuronal death and delayed neuronal recovery in the human brain following global ischemia. Acta Neuropathol Berl 85:79–87
10. Safar P (1993) Cerebral resuscitation after cardiac arrest: research initiatives and future directions. Ann Emerg Med 22:324–349
11. Fischer M, Hossmann K-A (1995) No-reflow after cardiac arrest. Intensive Care Med 21:132–141
12. Hossmann K-A, Fischer M, Bockhorst K, Hoehn-Berlage M (1994) NMR imaging of the apparent diffusion coefficient (ADC) for the evaluation of metabolic suppression and recovery after prolonged cerebral ischemia. J Cereb Blood Flow Metab 14:723–731
13. Fischer EG, Ames A, Hedley-Whyte ET, O'Gorman S (1977) Reassessment of cerebral capillary changes in acute global ischemia and their relationship to the "no-reflow phenomenon". Stroke 8:36–39
14. Lin SR (1979) Changes of plasma volume and hematocrit after circulatory arrest: an experimental study in dogs. Invest Radiol 14:202–206
15. Hekmatpanah J (1973) Cerebral blood flow dynamics in hypotension and cardiac arrest. Neurology 23:174–180
16. Hossmann K-A, Hossmann V (1977) Coagulopathy following experimental cerebral ischemia. Stroke 8:249–254
17. Hossmann V, Hossmann K-A, Takagi S (1980) Effect of intravascular platelet aggregation on blood recirculation following prolonged ischemia of the cat brain. J Neurol 222:159–170
18. Gaszynski W (1974) Research work on blood clotting system during cardiorespiratory resuscitation. Anaesth Resusc Intensive Ther 2:303–316
19. Hossmann K-A (1988) Resuscitation potentials after prolonged global cerebral ischemia in cats. Crit Care Med 16:964–971
20. Stullken EH, Sokoll MD (1976) The effect of heparin on recovery from ischemic injuries in cats. Anesth Analg 55:683–687
21. Fischer EG, Ames A (1972) Studies on mechanical impairment of cerebral circulation following ischemia: effect of hemodilution and perfusion pressure. Stroke 3:538–542

22. Arnout J, Simoons M, de-Bono D, Rapold HJ, Collen D, Verstraete M (1992) Correlation between level of heparinization and patency of the infarct-related coronary artery after treatment of acute myocardial infarction with alteplase (rt-PA). J Am Coll Cardiol 20:513–519
23. Theilen J, Schrock H, Kuschinsky W (1993) Capillary perfusion during incomplete forebrain ischemia and reperfusion in rat brain. Am J Physiol 265: H642–H648
24. Korninger C, Collen D (1981) Studies on the specific fibrinolytic effect of human extrinsic (tissue-type) plasminogen activator in human blood and in various animal species in vitro. Thromb Haemost 46:561–565
25. Iijima T, Bauer R, Hossmann K-A (1993) Brain resuscitation by extracorporeal circulation after prolonged cardiac arrest in cats. Intensive Care Med 19:82–88
26. Wolfson S Jr, Safar P, Reich H, Clark JM, Gur D, Stezoski W, Cook EE, Krupper MA (1992) Dynamic heterogeneity of cerebral hypoperfusion after prolonged cardiac arrest in dogs measured by the stable xenon/CT technique: a preliminary study. Resuscitation 23:1–20
27. Safar P, Abramson NS, Angelos M, Cantadore R, Leonov Y, Levine R, Pretto E, Reich H, Sterz F, Stezoski SW, Tisherman S (1990) Emergency cardiopulmonary bypass for resuscitation from prolonged cardiac arrest. Am J Emerg Med 8:55–67
28. Nakayama S, Sibley L, Gunther RA, Holcroft JW, Kramer GC (1984) Small-volume resuscitation with hypertonic saline (2,400 mOsm/liter) during hemorrhagic shock. Circ Shock 13: 149–159
29. Messmer K, Kreimeier U (1989) Microcirculatory therapy in shock. Resuscitation 18:51–61
30. Fischer M, Hossmann K-A (1996) Volume expansion during cardiopulmonary resuscitation reduces cerebral no-reflow. Resuscitation (in press)
31. Strecker U, Dick W, Heimann A, Kempski O (1993) Hypertonic-hyperoncotic HES improves outcome from global cerebral ischemia. Anesthesiology 79: A577
32. Dirnagl U, Niwa K, Sixt G, Villringer A (1994) Cortical hypoperfusion after global forebrain ischemia in rats is not caused by microvascular leukocyte plugging. Stroke 25:1028–1038
33. Hallenbeck JM (1994) Blood-damaged tissue interaction in experimental brain ischemia. Acta Neurochir Suppl 60: 233–237
34. Mori E, del-Zoppo GJ, Chambers JD, Copeland BR, Arfors KE (1992) Inhibition of polymorphonuclear leukocyte adherence suppresses no-reflow after focal cerebral ischemia in baboons. Stroke 23:712–718
35. Crowell JW, Sharpe GP, Lambright RL, Read WL (1955) The mechanism of death after resuscitation following acute circulatory failure. Surgery 38: 696–702
36. Crowell JW, Smith EE (1956) Effect of fibrinolytic activation on survival and cerebral damage following periods of circulatory arrest. Am J Physiol 186: 282–285
37. Gaszynski W (1975) The use of protease inhibitor (trasylol) and heparin in cardiorespiratory resuscitation. I. Studies of the blood clotting system. Anaesth Resusc Intensive Ther 3: 125–134
38. Gaszynski W (1975) Use of protease inhibitor (trasylol) and heparin in cardiorespiratory resuscitation. II. Gasometric investigations of arterial blood. Anaesth Resusc Intensive Ther 3: 203–311
39. Lin SR, O'Connor MJ, Fischer HW, King A (1978) The effect of combined dextran and streptokinase on cerebral function and blood flow after cardiac arrest: an experimental study on the dog. Invest Radiol 13:490–498
40. Safar P, Stezoski W, Nemoto EM (1976) Amelioration of brain damage after 12 minutes' cardiac arrest in dogs. Arch Neurol 33:91–95
41. Zimmermann V, Hossmann V, Hossmann K-A (1975) Intracranial pressure after prolonged cerebral ischemia. In: Lundberg N, Pontén U, Brock M (eds) Intracranial pressure II. Springer, Berlin Heidelberg New York, pp 177–182
42. Vise WM, Schuier F, Hossmann K-A, Takagi S, Zülch KJ (1977) Cerebral microembolization. I. Pathophysiological studies. Arch Neurol 34:660–665
43. Böttiger BW, Motsch J, Böhrer H, Böker T, Aulmann M, Nawroth PP, Martin E (1995) Activation of blood coagulation after cardiac arrest is not balanced adequately by activation of endogenous fibrinolysis. Circulation 92:2572–2578
44. Tenaglia AN, Califf RM, Candela RJ, Kereiakes DJ, Berrios E, Young SY, Stack RS, Topol EJ (1991) Thrombolytic therapy in patients requiring cardiopulmonary resuscitation. Am J Cardiol 68:1015–1019
45. Scholz KH, Tebbe U, Herrmann C, Wojcik J, Lingen R, Chemnitius JM, Brune S, Kreuzer H (1992) Frequency of complications of cardiopulmonary resuscitation after thrombolysis during acute myocardial infarction. Am J Cardiol 69:724–728
46. Böttiger BW, Böhrer H, Bach A, Motsch J, Martin E (1994) Bolus injection of thrombolytic agents during cardiopulmonary resuscitation for massive pulmonary embolism. Resuscitation 28: 45–54
47. Weston CF, Avery P (1992) Thrombolysis following pre-hospital cardiopulmonary resuscitation. Int J Cardiol 37:195–198