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ABSTRACT

The iron chelating agent deferoxamine was studied in an animal model as post-resuscitation therapy to prevent late deaths and brain damage following total circulatory arrest and resuscitation. Cardio-respiratory arrest was induced by injection of cold, 1% KC1 into the left ventricles of ketamine anesthetized rats pretreated with succinylcholine chloride, and by discontinuation of positive pressure ventilation. CPR was begun after six minutes, and animals with return of spontaneous circulation were entered into the study. Within five minutes after return of spontaneous circulation, treated animals received deferoxamine (50 mg/kg, IV). At ten days, 16 of 25 (64%) of treated animals had survived without neurologic deficit, compared to nine of 25 (36%) of controls (chi square = 3.92, $P < .05$). Chelation of intracellular iron by deferoxamine may have prevented free-radical-mediated reactions that led to late deaths in control animals.

Key words: animal model, cardiac arrest, reactive oxygen species, sudden death, ventricular fibrillation

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INTRODUCTION

Late deaths following initially successful CPR may be related to specific and preventable phenomena that occur during the reperfusion period [1-4]. Indeed, a large proportion of the total injury seen after five-minute to 15- minute periods of circulatory arrest may develop during the reperfusion phase, due to 1) the no-reflow phenomenon [5], in which cerebral vascular resistance rises during reperfusion after ischemic anoxia, decreasing perfusion to areas of the brain; 2) continued calcium influx through cell membranes damaged during anoxia, leading to intracellular calcium intoxication during reperfusion [2, 3, 6, 7]; or 3) production of oxidative free radicals, causing progressive lipid peroxidation in cell membrane systems, particularly in the lipid-rich brain [l, 3, 8]. If, in fact, irreversible damage to the brain and other organs by such mechanisms occurs during the period of reperfusion rather than during the period of anoxia, the corresponding pathophysiologic entity, "reperfusion injury" ,may be treatable as part of advanced cardiac life support (ACLS).

Deferoxamine is a clinically available iron-binding agent with an extremely high affinity for iron. It is water soluble, yet crosses the blood brain barrier, and does not interfere significantly with the oxygen transport function of hemoglobin [9]. Blaine C. White [3] recently proposed that free iron ions liberated from intracellular stores during ischemic anoxia may play a major role in the genesis of reperfusion injury by oxidative free radicals. Our study was designed as a preliminary test of the potential of deferoxamine to prevent late deaths following initially successful CPR.

MATERIALS AND METHODS

The rat circulatory arrest model used has been described previously [10]. Fifty male Wistar rats, each weighing between 350 and 450 g, were sorted randomly into experimental and control groups of 25 animals each. Each rat was anesthetized with ketamine 160 mg/kg intraperitoneally (IP). Succinylcholine chloride 1.5 mg/kg, IP was given to prevent gasping at the onset of circulatory arrest. A midline tracheostomy was performed, and jet ventilation with room air at 40 breaths per minute was provided through a teflon cannula by a Physiograph® small animal respirator (Narco Bio-Systems, Houston, Texas).

Cardiorespiratory arrest was induced in each rat by discontinuation of jet ventilation, percutaneous intracardiac injection of 0.8 mL cold 1% KCl cardioplegic solution, and steady digital compression of the thorax [10]. The ECG was monitored throughout the arrest and immediate post-resuscitation periods. The cardio-respiratory arrest was maintained for six minutes, after which resuscitation was accomplished by interposed abdominal compression-cardiopulmonary resuscitation (IAC-CPR) and jet ventilation with room air at 70 breaths per minute for one to four minutes.

Animals in which sinus rhythm and a palpable heartbeat returned were entered into either the experimental or control groups. Rats in the experimental group were given deferoxamine (50 mg/kg) through an external jugular vein during a one-minute period. (Deferoxamine (Desferal®) was obtained as a powder from CIBA Pharmaceutical Company, Summit, New Jersey, and was dissolved freshly in de-ionized water to a concentration of 50 mg/mL.) The control rats were given no drug.

All rats then were placed on a 37 ^{o}C heating pad and given 5 mL each 5% dextrose solution and lactated Ringer's solution by subcutaneous injection to maintain hydration. The rats were weaned from the respirator by gradual decrease in the ventilation frequency to 20 breaths per minute during a period of one to two hours. When vigorous spontaneous respirations were observed, jet ventilation was discontinued, the trachea was extubated, and the neck wound was closed with silk sutures.

Thereafter the rats were returned to individual cages where they were observed daily for ten days, during which no further intensive care was provided. Neurologic deficit score was measured at 24 hours after arrest and daily thereafter for seven days, or until death, by a modification of the method of Safar and coworkers [11] as adapted for rats by deGaravilla [12]. The number of animals surviving was recorded at 12 hours, 24 hours, and daily for ten days. Chi-square statistics were calculated to test the null hypothesis that at each recording time the percentage of rats surviving in the control and treated groups was the same.

RESULTS

Most resuscitated rats could be weaned from the ventilator within one to two hours after resuscitation. At three to six hours after arrest, survivors in both groups were awake but ataxic, with depressed responsiveness to touch, painful stimuli, and air jets.

Deaths during the first 12 hours were associated with failure to recover from anesthesia or to be weaned from the ventilator. At 36 hours, however, all survivors were awake and responsive, without localized neurologic deficit. Although the mean neurologic deficit score of survivors at 36 hours was zero in both treated and control groups, animals destined to die in the second through fourth days after arrest huddled in the comers of their cages and became progressively anorexic, dehydrated, and less well groomed. In contrast, those destined to survive long-term remained alert, active, and well groomed, and they began to eat and drink normally.

At ten days after arrest, surviving animals in both treated and control groups were without neurologic deficit. The incidence of survival in the treated group, however, was significantly greater than in the control group at days 7 through 10 (chi square = 3.92; $0.01 < P < 0.05$). At the end of the ten-day period, survival rates for the control and experimental groups were 36% and 64%, respectively. Trends in survival as a function of time are shown (Figure).

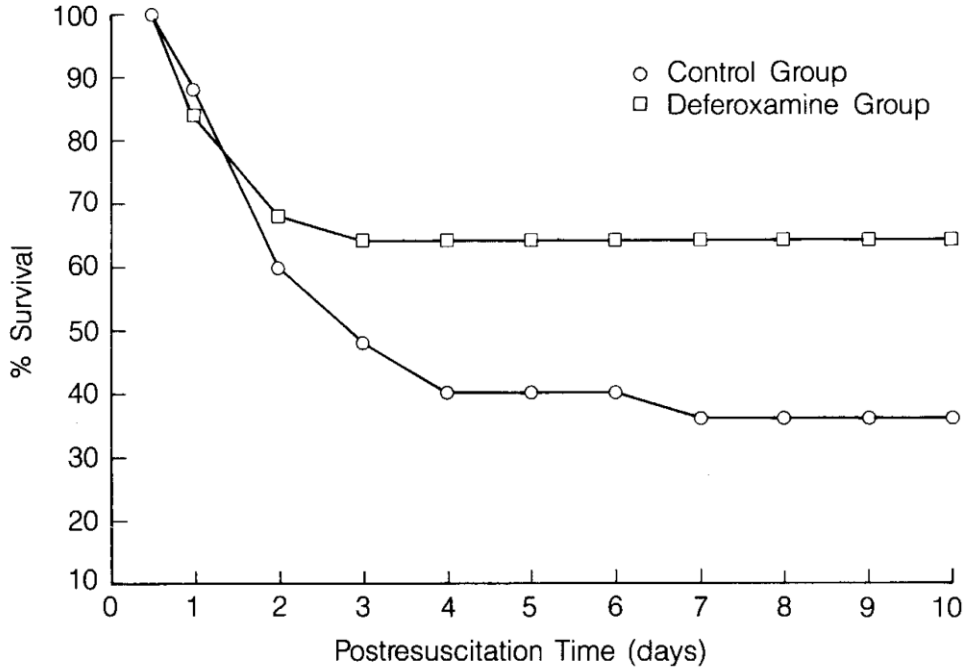

FIGURE. Survival as a function of time after cardiac arrest and resuscitation in deferoxamine-treated versus control rats. The deferoxamine dose was 50 mg/kg IV, and each group comprised 25 animals.

DISCUSSION

Our study found a statistically significant increase in long-term survival following seven-minute to ten-minute periods of total circulatory arrest and CPR in a whole animal model of ischemia and reperfusion in rats. To place these results in perspective, one should consider that since the discovery of modem CPR methods in the 1960s, the only interventions shown to produce comparable improvement in ***long-term*** survival in any animal model or in human beings have been the use of adrenergic drugs such as epinephrine [13, 14] and the use of earlier electrical defibrillation in cases of ventricular fibrillation [15, 16].

It is of particular interest that survival curves separate only after the second day. This observation, together with the differing behavior of survivors and non-survivors during the second through fourth days, is consistent with the possibility of gradually developing tissue injury during the first few days after reperfusion. Such a time course is consistent with that of ongoing injury by iron-catalyzed lipid peroxidation. Recent unpublished computer simulations of the kinetics of lipid peroxidation reactions suggest that such reactions may continue and even accelerate for hours to days after their initiation (Charles F Babbs, MD, unpublished data). Thus

the time course of the survival curves is consistent with the type of smoldering oxidative reactions that we have proposed as a biochemical mechanism for post-resuscitation brain injury [4].

Our study, however, is quite limited as a test of the lipid peroxidation hypothesis. The task remains to search for biochemical evidence of lipid peroxidation in this model and the diminution of lipid peroxidation in deferoxamine-treated animals. Further, it will be important in the future to test control animals treated with iron-loaded deferoxamine (i.e., ferioxamine) to determine if some action of the drug other than its iron-binding capability is responsible for the protective effect.

CONCLUSION

Our study clearly is a preliminary one, and we do not recommend the clinical use of iron chelators until their mechanism of action and safety have been established in further animal studies. Nevertheless, because deferoxamine administered ***after*** return of spontaneous circulation significantly improved long-term survival, the results are consistent with the hypothesis that a substantial amount of preventable tissue damage, leading to death, was occurring in the control animals during the reperfusion phase, and that the mechanism of damage involves iron ions.

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REFERENCES

1. McCord JM: Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med* 1985;312:159-163.

2. White BC, Winegar CD, Wilson RF, et al: Possible role of calcium blockers in cerebral resuscitation: A review of the literature and synthesis for future studies. *Crit Care Med* 1983;11:202-207.

3. White BC, Aust SD, Arfors KE, et al: Brain injury by ischemic anoxia: Hypothesis extension -- a tale of two ions? *Ann Emerg Med* 1984;13:862-867.

4. Babbs CF, "Role of iron i6ns in the genesis of reperfusion injury following successful cardiopulmonary resuscitation: Preliminary data and a biochemical hypothesis. *Ann Emerg Med* 1985;14:777-783.

5. Ames A, Wright L, Kowada M, et al: Cerebral ischemia; II. The no-reflow phenomenon. *Am J Pathol* 1968;52: 437-438.

6. Farber JL: Minireview: The role of calcium in cell death. *Life Sciences* 1981;29:1289-1295.

7. Babbs CF: Improved methods of resuscitation from sudden cardiac death -- research opportunities for emergency physicians. *Emergency Medicine Survey* 1982;103-108.

8. McCord JM: The superoxide free radical, its biochemistry and pathophysiology. *Surgery* 1983;94:412-414.

9. Keberle H: The biochemistry of desferrioxamine and its relation to iron metabolism. *Ann NY Acad Sci* 1974;119:758-768.

10. deGaravilla L, Babbs CE Tacker WA: An experimental circulatory arrest model in the rat to evaluate calcium antagonists in cerebral resuscitation. *Am J Emerg Med* 1984;2:321-326.

11. Safar P, Stezoski W, Nemoto EM: Amelioration of brain damage after 12 minutes cardiac arrest in dogs. *Arch Neurol* 1976;33:91-95.

12. deGaravilla L: Effect of diltiazem on brain resuscitation in a rat circulatory arrest model. PhD thesis, Purdue University, August 1984.

13. Pearson JW, Redding JS: The role of epinephrine in cardiac resuscitation. *Anesth Analg* 1963;42:599-606.

14. Otto CW, Yakaitis RW, Redding JS, et al: Comparison of dopamine, dobutamine, and epinephrine in CPR. *Crit Care Med* 1981;9:366.

15. Eisenberg MS, Bergner L, Hallstrom A: Cardiac resuscitation in the community. *JAMA* 1979;241:1905-1907.

16. Eisenberg MS, Bergner L, Hallstrom A: Out-of-hospital cardiac arrest: Improved survival with paramedic services. *Lancet* 1980;812-815.