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# THE EFFECT OF CARBON DIOXIDE, LIDOFLAZINE, AND DEFEROXAMINE UPON LONG TERM SURVIVAL FOLLOWING CARDIORESPIRATORY ARREST IN RATS

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## SUMMARY

This study examined the effect of carbon dioxide, lidoflazine, and deferoxamine therapy upon the 10-day survival incidence and subsequent neurologic function of rats subjected to 7 min of cardiorespiratory arrest with resuscitation. Cardiac arrest (asystole) was induced at time zero by injection of cold, 1% KCl into the left ventricle of ketamine-anesthetized rats pretreated with succinylcholine. Positive pressure ventilation was discontinued at time zero. Cardiopulmonary resuscitation (CPR) was begun at 7 min, and animals with return of spontaneous circulation were entered into the study. Twenty treated rats were ventilated for 1 h with 7% carbon dioxide-93% oxygen and given lidoflazine (2.0 mg/kg, i.v.) and deferoxamine (50 mg/kg, i.v.) 5 min after CPR. Twenty control rats were ventilated for 1 h with 100% oxygen and given lidoflazine vehicle and deferoxamine vehicle. Lidoflazine treatment (1.0 mg/kg) for the treated group, or lidoflazine vehicle for the control group, was repeated at 8 h postresuscitation. At 2 days postresuscitation, 75% of treated rats vs. 25% of control rats were alive (Chi-square = 10.0, d.f. = 1,  $P < 0.01$ ), and at 10 days, 60% of treated rats vs. 25% of control rats were alive (Chi-square = 5.01, d.f. = 1,  $P < 0.05$ ). There was no detectable neurologic deficit among survivors in either group at 15 days. The combination of carbon dioxide, lidoflazine, and deferoxamine, administered after return of spontaneous circulation, is a simple and easily administered treatment regimen that improves the survival incidence without neurologic deficits in this animal model of cardiorespiratory arrest and CPR.

**Key words:** Cardiorespiratory arrest - Carbon dioxide - Lidoflazine - Deferoxamine- Survival rate- Neurologic deficit

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**Abbreviations:** CPR, cardiopulmonary resuscitation; IAC-CPR, interposed abdominal compression-cardiopulmonary resuscitation.

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## INTRODUCTION

Modern methods of cardiopulmonary resuscitation and advanced cardiac life support can successfully restore spontaneous circulation in up to 56% of victims of prehospital cardiac arrest (DeBard, 1981). However, fewer than half of the initially resuscitated patients survive to leave the hospital (DeBard, 1981; Eisenberg et al., 1979; Myerburg et al., 1980). Central nervous system (CNS) damage and hemodynamic abnormalities have caused 59% and 31%, respectively, of postresuscitation, in-hospital deaths (Myerburg et al., 1980), and permanent functional neurologic disability is reported in 10%-40% of patients surviving prehospital cardiac arrest (Liberthson et al., 1974). In recent years, evidence has begun to accumulate to support the hypothesis that a significant fraction of the irreversible tissue injury observed after ischemia and reperfusion is actually caused during the reperfusion phase, and is therefore amenable to prevention or treatment by therapy administered after initially successful cardiac resuscitation, both in the emergency room and during subsequent hospitalization.

Selected therapeutic interventions directed at one or more of the changes which have been postulated to occur after resuscitation from cardiac arrest, or have been shown *in vitro* to occur during reperfusion of ischemic tissue, may be a logical means of preventing or minimizing the adverse effects that occur during the postresuscitation period. In the present study, we evaluated the effectiveness of a simple and easily administered multi-modal therapy, including a cerebral vasodilator (carbon dioxide), a calcium entry blocker (lidoflazine), and an iron chelator (deferoxamine), intended to minimize reperfusion injury following total circulatory arrest and CPR in rats. All therapy was administered after return of spontaneous circulation in a clinically realistic time frame (DeGaravilla et al., 1984).

## MATERIALS AND METHODS

Male Wistar rats, each weighing between 350 and 500 g, were utilized in this study. Following anesthesia with an intraperitoneal injection of ketamine (60 mg/kg) and neuromuscular blockade with succinylcholine (1.5 mg/kg), cardiorespiratory arrest (asystole) was induced in each rat by a percutaneous intracardiac injection of 0.4-0.8 ml of a cold 1% KCl cardioplegic solution and digital compression of the thorax (DeGaravilla et al., 1984). Electrocardiograms were monitored throughout the arrest and immediate postresuscitation period. The cardiorespiratory arrest was maintained for 7 min. Resuscitation was accomplished by interposed abdominal compression-cardiopulmonary resuscitation (IAC-CPR) (Voorhees et al., 1984) and jet ventilation at 70 breaths/min with 7% carbon dioxide-93% oxygen (treated group) or with 100% oxygen (control group). The ventilatory gas used, and therefore the group entered, was alternated each time an animal was successfully resuscitated and entered into the study. Any variability in handling the animals and in the procedures used was effectively minimized in this fashion. This method of cardiorespiratory arrest and resuscitation was continued until 20 rats each were successfully resuscitated and entered both groups.

Following 1-3 min of IAC-CPR, the return of a strong spontaneous apex heartbeat, and the return to sinus rhythm as documented by the electrocardiogram, the treated rats were given lidoflazine (2 mg/kg) and deferoxamine (50 mg/kg) by slow intravenous infusion for a 15-min period. The control rats were given equivalent volumes of the vehicle for the lidoflazine solution (a dilute acetic acid solution, pH = 4.5) and the solvent for the deferoxamine (0.9% saline) by jugular intravenous infusion. Lidoflazine (1.0 mg/kg) was given to treated rats again at 8 h postresuscitation by intraperitoneal injection, and control rats received the lidoflazine vehicle. All rats were placed on a 37°C heating pad after resuscitation, and 5 ml each of 5% dextrose solution and lactated ringers solution were given by subcutaneous injection to maintain hydration. The ventilation frequency was gradually reduced to 20/min during the subsequent 120 minutes and then discontinued when vigorous spontaneous respirations were observed.

The rats were returned to individual cages following resuscitation and stabilization, and were observed daily. The number of animals surviving postresuscitation was recorded at 1 h, 12 h, 24 h, and at daily intervals for 10 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 (Dunn, 1977).

Neurologic deficits at 15 days postresuscitation were evaluated in each rat by applying a modification of the Safar neurologic scoring method for dogs (Safar et al., 1976). This method has been validated in numerous studies in this laboratory and has proven applicable to the rat model of cardiorespiratory arrest and resuscitation. All neurologic evaluations were done by the same individual and without knowledge of the treatment group to which the rat belonged. The specific tests and the corresponding point values are listed in Table I. A score of 0 represented no detectable neurologic deficits, and a score of 100 points represented maximal neurologic deficit (brain death).

## TABLE I. NEUROLOGIC DEFICIT SCORING CRITERIA

Neurologic deficit scoring for rats: Normal rat = 0, brain death = 100.  
Modified from the neurological deficit scoring for dogs from Safar and Nemoto.

### Level of consciousness

Normal = 0, Clouded = 2, Delerium = 4, Coma = 8

### Reflexes and motor function

Pupil size: normal = 0, slightly dilated = 2, dilated = 4

Pupillary reaction to light: normal = 0, sluggish = 2, absent = 4

Extensor reflex: normal = 0, sluggish = 2, absent = 4

Corneal reflex: normal = 0, sluggish = 2, absent = 4

Response to touch: normal = 0, sluggish = 2, absent = 4

Response to pain: normal = 0, sluggish = 2, absent = 4

Response to burst of air: normal = 0, sluggish = 2, absent = 4

Muscle tone: normal = 0, slightly flaccid = 2, absent = 4

Paralysis: normal = 0, partial = 2, total = 4

Righting reflex: normal = 0, present but sluggish = 2,  
absent with feeble attempts = 4, absent = 6

Inclined plane response: normal = 0, sluggish = 2,  
absent with feeble attempts = 4, absent = 6

Spontaneous locomotor activity (open field): normal = 0,  
reduced = 3, absent = 6

Ataxia: normal = 0, partial (2 limbs or less) = 3, severe = 6

### Miscellaneous

Defecation: normal = 0, absent = 2

Response to noise: normal = 0, diminished = 2, absent = 4

Sniffing and whisker activity: normal = 0, diminished = 2, absent = 4

Ability to stand: normal = 0, diminished = 2, absent = 4

Ability to drink: normal = 0, absent = 2

Ability to eat: normal = 0, absent = 2

Grooming behavior: normal = 0, absent = 2

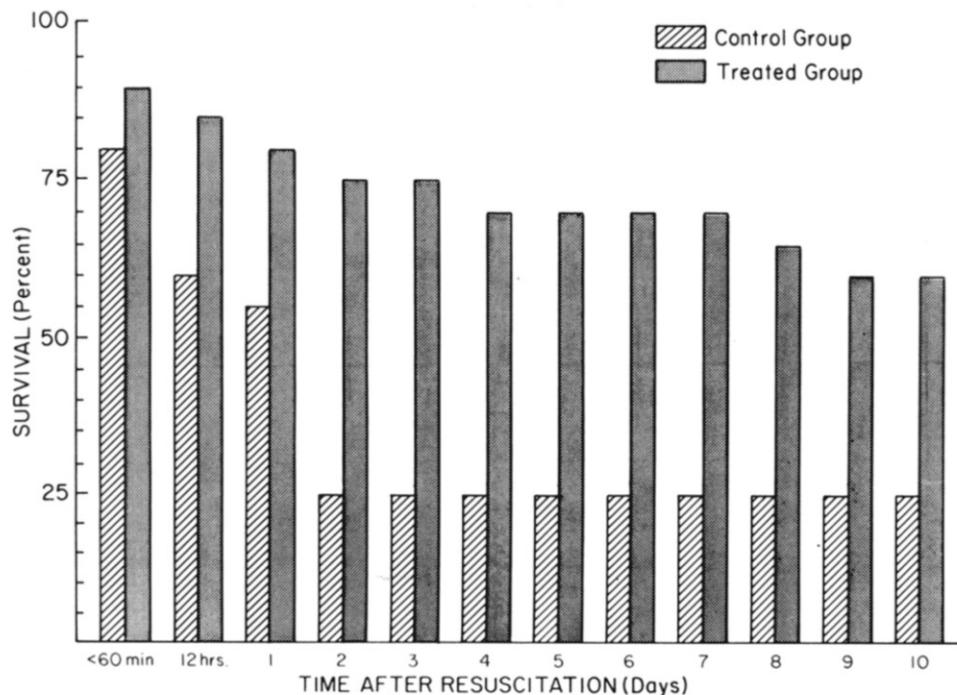
### Respiration

Rate: normal = 0, decreased/increased = 3, absent = 6

Pattern: normal = 0, labored = 3, gasping = 4, absent = 6

## RESULTS

Eighty-four percent of the rats (20/24) in which 100% oxygen was the ventilatory gas (control group) were successfully resuscitated and entered in the study, and 77% of the rats (20/26) in which 93% carbon dioxide/7% oxygen was the ventilatory gas (treated group) were successfully resuscitated and entered into the study. Rats in the control group regained a spontaneous sinus rhythm and palpable heart beat in a mean time of 74 sec (range of 62-90 sec, median of 72 sec). Rats in the treated group regained a spontaneous sinus rhythm and palpable heart beat in a mean time of 76 sec (range of 60-83 sec, median of 69 sec). The percentage of rats surviving was significantly greater (Chi-square = 10.0, d.f. = 1,  $P < 0.01$ ) in the treated group than the control group, 75% vs. 25% respectively, at 2 days postresuscitation (Fig. 1). The difference in survival between the 2 groups was maximum at 2 and 3 days postresuscitation. After 3 days, the difference in survival between the 2 groups remained relatively constant, and after 10 days 60% of the treated animals were alive and 25% of the control animals were alive (Chi-square = 5.0, d.f. = 1,  $P < 0.05$ ).



**Fig. 1. The sequential survival incidence of treated vs. control rats for 10 days following resuscitation from 7 min of cardiorespiratory arrest ( $n = 20$  for each group).**

It should be noted that lidoflazine frequently caused bradycardia and a palpable diminution of the apex beat if given during less than a 15-min time span. Neurologic deficit scores were not different between the treated and control groups. The mean score for survivors in both the treated and control groups was 2 out of a possible 100 points. The ranges of scores for survivors in the treated and control groups were 0-4 and 0-3, respectively.

## DISCUSSION

Increased awareness of cardiopulmonary resuscitation techniques by the general public, and the development of community based emergency medical systems have increased the number of patients that survive an out-of-hospital cardiac arrest (Myerberg et al., 1980; Liberthson et al., 1974). However, many of these patients either die within the early postresuscitation period or survive with major organ disability, especially CNS dysfunction (Liberthson et al., 1974; Schaeffer and Cobb, 1975).

The rationale for utilizing carbon dioxide, lidoflazine and deferoxamine in the present study to treat ischemic injury was based upon our current understanding of the pathophysiology of this type of injury in various tissues, previous studies in our laboratory, and the known effects of these drugs in normal tissues. First, Ames' "no reflow phenomenon" (Ames et al., 1968), which describes postischemic cerebral hypoperfusion, has been well substantiated by others (Gadzinski et al., 1982; Rehnrona et al., 1979; Snyder et al., 1973). This phenomenon may be caused by progressively increased cerebral vascular resistance without changes in intracranial pressure or evidence of thrombosis (Gadzinski et al., 1982; Rehnrona et al., 1979; Drewes et al., 1973). Therefore, maintenance of cerebral blood flow during the reperfusion phase after cardiac arrest appears to be a limiting factor in cerebral recovery from ischemic injury. Carbon dioxide is an effective and physiologic cerebral vasodilator which may alleviate postresuscitation global hypoperfusion of the brain (Cook and James, 1981; Harper, 1969).

Second, several lines of evidence strongly implicate calcium in the pathogenesis of postischemic injury in both neural and non-neural tissue (Katz and Reuter, 1979; Schaune et al., 1979; Farber et al., 1981; Raichle, 1983). Three separate, potentially cytotoxic events have been attributed to intracellular calcium accumulation during reperfusion: (1) spasm of vascular smooth muscle, (2) mitochondrial failure, and (3) activation of intracellular membrane phospholipases. Lidoflazine, a calcium entry blocker, may inhibit the vasospasm and the intracellular calcium overload that occur following ischemia and reperfusion (Daenen and Flameng, 1981). This drug and other calcium antagonists have been reported to decrease neurologic deficits and cardiac pathologic alterations in animals following resuscitation from cardiac arrest (Daenen and Flameng, 1981; Hoffmeister et al., 1979; White et al., 1982; Winegar et al., 1983).

Finally, membrane lipid peroxidation by free radicals has been proposed as a major mechanism for reperfusion injury (Siesjo et al., 1980; Cooper et al., 1980; Watson et al., 1984). Iron-containing enzymes (e.g. xanthine oxidase, lipoxygenase) and ferrous chelate complexes (e.g. EDTA-Fe<sup>2+</sup>, AMP-Fe<sup>2+</sup>) have been associated with the initiation of lipid peroxidation (Aust et al., 1982; Bucher et al., 1983; Sugioka et al., 1983). Anderson and Means have shown that iron and oxidative mechanisms are important factors in traumatic spinal cord injury in the cat (Anderson and Means, in press, 1986). Oxygen radicals have not been demonstrated in ischemic tissue however, and the status of intracellular protective mechanisms against free radicals has been the subject of conflicting reports (Siesjo et al., 1980; Cooper et al., 1980). Deferoxamine, a safe, clinically available iron binding agent, inhibits lipid peroxidation in vitro, and administration of this drug to rats following cardiopulmonary arrest has resulted in increased survival compared to untreated control rats in our laboratory (unpublished data).

The improved survival incidence that we found in animals treated with carbon dioxide, lidoflazine, and deferoxamine suggests that one or more of the biologic changes induced by ischemia and reperfusion, and altered or inhibited by these drugs, is a critical determinant in the progression to irreversible tissue injury during reperfusion. The marked difference between the treated and control groups at 2 days postresuscitation suggests that a critical step in the progression to irreversible tissue injury occurred at this time and that our therapeutic intervention had a beneficial effect upon host survival. In addition, one may speculate that the continued death of rats in the treated group is due to progressive reperfusion injury that occurred in animals that would have succumbed early following resuscitation but were protected by the combination therapy. The reperfusion tissue damage in these animals then progressed to lethal levels as the effects of the discontinued therapy diminished. The blood pressure was not monitored in the rats during this study. Therefore, the potential effect upon survival of changes in this parameter following therapy cannot be addressed.

The neurologic deficit evaluation showed that all surviving animals were grossly normal, indicating that the quality of survival among treated animals was no different than that among controls. In addition, this combination therapy does not prolong recovery nor have detectable adverse side effects in this animal model. No attempt was made in this study to delineate the individual effectiveness of each therapeutic agent. The observed effects may have been due to any one, or combination of more than one, of the therapeutic modalities. Therefore, a part of or all of the combination therapy consisting of a cerebral vasodilator, a calcium entry blocker, and an iron chelator represents a feasible means of protection from postresuscitation morbidity and mortality following cardiorespiratory arrest that merits further investigation.

## REFERENCES

- Ames, A.,III, Wright, R.L. and Kowada, M. et al. (1968) Cerebral ischemia: II. The no reflow phenomenon. *Am. J. Pathol.*, 52, 437-453.
- Anderson, D.K. and Means, E.D. (1986) Lipid peroxidation in spinal cord: FeCl<sub>3</sub>, induction and antioxidants. *Neurochem. Pathol.*, in press.
- Aust, S.D. and Svingen, B.A. (1982) The role of iron in enzymatic lipid peroxidation. *Free Radicals in Biology*, 5, 1-28.
- Bucher, J.R., Tien, M. and Aust, S.D. (1983) The requirement for ferric in the initiation of lipid peroxidation by chelated ferrous iron. *Biochem. Biophys. Acta*, 111,777-784.
- Cook, P. and James, I. (1981) Cerebral vasodilators. *N. Engl. J. Med.*, 305, 1508-1513, 1560-1564.
- Cooper, A.J., Pulsineli, W.A. and Duffy, T.F. (1980) Glutathione and ascorbate during ischemia and postischemic reperfusion in the rat brain. *J. Neurochem.*, 35, 1242-1245.

- Daenen, W. and Flameng, W. (1981) Myocardial protection by lidoflazine during one-hour normothermic global ischemia. *Angiology*, 32, 543-548.
- DeBard, M.L. (1981) Cardiopulmonary resuscitation: Analysis of six years' experience and review of the literature. *Ann. Emerg. Med.*, 10, 408-416.
- DeGaravilla, L., Babbs, C.F. and Tacker, W.A. (1984) An experimental circulatory arrest model in the rat to evaluate calcium antagonists in cerebral resuscitation. *Am. J. Emerg. Med.*, 2, 321-326.
- Drewes, L.R., Gillia, D.D. and Betz, A.L. (1973) Metabolic alterations in brain during anoxic-anoxia and subsequent recovery. *Arch. Neurol.*, 293, 385-395.
- Dunn, O.J., Editors (1977) *Basic Statistics: A Primer for the Biomedical Sciences*, pp. 122-135. John Wiley and Sons, New York.
- Eisenberg, M.S., Bergner, L. and Hallstrom, A. (1979) Cardiac resuscitation in the community: Importance of rapid provision and implications for program planning. *J. Am. Med. Assoc.*, 241, 1905-1907.
- Farber, J.L., Chien, K.R. and Mitnacht, S., Jr. (1981) The pathogenesis of irreversible cell injury in ischemia. *Am. J. Pathol.*, 102, 271-281.
- Gadzinski, D.S., White, B.C. and Hoehner, P.J. et al. (1982) Alterations in canine cerebral cortical blood flow and vascular resistance post cardiac arrest. *Ann. Emerg. Med.*, 11, 58-63.
- Harper, A.M. (1969) Regulation of cerebral circulation. In: *Scientific Basis of Medicine Annual Review: British Post Graduate Medical Federation*. pp. 60-81. Athlone Press, London.
- Hoffmeister, F., Kazda, S. and Krause, H.P. (1979) Influence of Nimodipine on the post-ischemic changes of brain function. *Acta Neurol. Scand.*, 56 (Suppl 72), 358-359.
- Katz, A.M. and Reuter, H. (1979) Cellular calcium and cardiac cell death. *Am. J. Cardiol.*, 44, 188-190.
- Liberthson, R., Nagel, E. and Hirschmann, J. (1974) Prehospital ventricular fibrillation: Prognosis and follow-up course. *N. Engl. J. Med.*, 291, 317-321.
- Myerburg, R.J., Conde, C.A. and Sung, R.J. et al. (1980) Clinical, electrophysiologic and hemodynamic profile of patients resuscitated from prehospital cardiac arrest. *Am. J. Med.*, 68, 568-576.
- Raichle, M.E. (1983) The pathophysiology of brain ischemia. *Ann. Neurol.*, 13, 2-10.
- Rehncrona, S., Abdul-Rohman, A. and Siesjo, B.K. (1979) Local cerebral blood flow in the post ischemic period. *Acta Neurol. Scand.*, 60 (Suppl), 294-295.

Safar, P., Stezoski, W. and Nemoto, E.M. (1976) Amelioration of brain damage after 12 minutes' cardiac arrest in dogs. *Arch. Neurol.*, 33, 91-99.

Schaeffer, W.A. and Cobb, L.A. (1975) Recurrent ventricular fibrillation and modes of death in survivors of out-of-hospital ventricular fibrillation. *N. Eng. J. Med.*, 293, 259-262.

Schaune, F.A.X., Kane, A.B. and Young, E.E. et al. (1979) Calcium dependence of toxic cell death: a final common pathway. *Science*, 206, 700-702.

Siesjo, B.K., Rehncrona, S. and Smith, D.S. (1980) Neuronal cell damage in the brain: Possible involvement of oxidative mechanisms. *Acta Physiol. Scand.*, 492 (Suppl), 121-128.

Snyder, J.V., Nemoto, E.M. and Carrol, R.G. et al. (1975) Global ischemia in dogs: Intracranial pressure, brain blood flow and metabolism. *Stroke*, 6, 21-29.

Sugioka, K., Nakano, H. and Nakano, M. et al. (1983) Generation of hydroxyl radicals during enzymatic reductions: Less involvement of hydroxyl radical and great importance of perferryl ion complexes in lipid peroxidation. *Biochem. Biophys. Acta*, 753, 411-421.

Voorhees, W.D., Ralston, S.H. and Babbs, C.F. (1984) Regional blood flow during cardiopulmonary resuscitation with abdominal counterpulsation in dogs. *Am. J. Emerg. Med.*, 2, 123-128.

Watson, B.D., Busto, R. and Goldberg, W.J. et al. (1984) Lipid peroxidation in vivo induced by reversible global ischemia in rat brain. *J. Neurochem.*, 42, 268-274.

White, B.C., Gadzinski, D.S. and Hoehner, P.J. et al. (1982) Effect of flunarizine on canine cerebral cortical blood flow and vascular resistance post cardiac arrest. *Ann. Emerg. Med.*, 11, 19-26.

Winegar, C.P., Henderson, O. and White, B.C. et al. (1983) Early amelioration of neurologic deficit by lidoflazine after fifteen minutes of cardiopulmonary arrest in dogs. *Ann. Emerg. Med.*, 12, 471-477.