Protection Against Irreversible Hemorrhagic Shock by Allopurinol (36854)

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The development of irreversible shock has long been considered to be primarily a circulatory insufficiency causing tissue anoxia. Evidence has accumulated indicating that one or more severe metabolic derangements may be responsible for the development of the irreversibility. Irreversible shock is produced in many situations such as hypovolemia, endotoxemia, trauma and burns resulting in inadequate blood flow to the tissues and gradual metabolic deterioration so that the subject's survival is no longer possible or at least in doubt.

Numerous reports indicate that a possible locus of metabolic derangement is in the high-energy phosphate system, a system which obtains energy primarily through oxidative processes. The energy is then used to drive various cellular functions. Jones, Crowell and Smith (1) have suggested that there is an increased production of uric acid during hypoxia of hemorrhage due to a net loss of purine derivatives which consequently limits the resynthesis of tissue ATP. Crowell, Jones and Smith (2) have also reported that the pretreatment of dogs with the xanthine oxidase inhibitor, allopurinol, prevented the rise in blood uric acid and increased the survival rate. He also reports that allopurinol plus hypoxanthine given following reinfusion of the shed blood resulted in an increased survival rate (2).

It has also been reported by Lefer, Dow and Berne (3) that allopurinol was ineffective in protecting against irreversible shock.

The purpose of the present experiments is to further test the efficacy of allopurinol in shock and to determine if the addition of other compounds that might facilitate ATP production would enhance survival.

Methods. Eighty mongrel dogs averaging 14 kg in weight were anesthetized with sodium pentobarbital (30 mg/kg iv). Additional doses were administered only if necessary to maintain an adequate level of anesthesia. The animal was loosely tied on his back and the trachea was intubated. Using aseptic procedures the right femoral artery and femoral vein were exposed. The animals were then administered heparin sodium (10 mg/kg). This dose was supplemented with 2 mg/kg doses each hour.

The right femoral artery was cannulated and connected to a reservoir bottle. The cannula was fitted with a sidearm to which was connected a Statham pressure transducer.

The animal was then allowed at least 60 min to stabilize following the above procedures. Arterial pressure was continuously monitored during this entire period. The animal was then allowed to hemorrhage into the reservoir until the mean arterial pressure reached 20 mm Hg. This pressure was then maintained by adjusting the reservoir height as needed throughout the period of hypotension. Since the reservoir was open to the circulation, as signs of circulatory failure developed, the animal could spontaneously take up the hemorrhaged blood as needed.

When the animal had taken up 10% of the blood in the reservoir (approaching irreversibility) atropine (0.5 mg/kg iv) was administered over at least a 10 min interval. If the infusion caused spontaneous uptake of blood from the reservoir the infusion rate was slowed in order to prevent the uptake from occurring. In those cases where uptake did occur the blood returned to the reservoir spontaneously soon after the atropine administration was completed. The purpose of the atropine

TABLE I.

Expt.	No. of animals	Survivors 24 hr	36 hr	48 hr	%
Group I (control) Dextrose/saline (10 ml/kg) + dextrose/saline (125 ml)	36	0	0	0	0
Group II Glucose/saline (10 ml/kg) Na allopurinol (75 mg/kg)	10	0	0	0	0
Group III Glucose/saline (10 ml/kg) Na allopurinol (75 mg/kg) hypoxanthine (40 mg/kg) adenine (8 mg/kg)	10	7	3	2	20
Group IV Dextrose/saline (10 ml/kg) inosine (8 mg/kg) alpha-ketoglutarate (100 mg/kg) oxalacetate (85 mg/kg)	10	3	3	2	20
Group V Same as Group III + inosine (8 mg/kg) alpha-ketoglutarate (100 mg/kg) oxalacetate (85 mg/kg)	14	10	9	6	43

was to reduce intestinal activity so that blood and fluid loss into this area will be minimized.

When 25% of the bled volume had been spontaneously taken from the reservoir by the dog the reservoir is raised to a height so that all the blood will reenter the dogs circulation.

At this point several modes of therapy were attempted. In Group I (controls, 36 animals) the animals received an intravenous infusion of 20 ml/kg of a 5.5% dextrose—0.9% saline solution. Group II (10 animals) received an infusion of 10 ml/kg glucose saline plus an infusion of sodium allopurinol (75 mg/kg). Group III (10 animals) received the same solutions as Group II with the addition of hypoxanthine (40 mg/kg) and adenine (8 mg/kg) Group IV, (10 animals) received 10 ml/kg glucose saline solution plus a solution containing inosine (8 mg/kg), alpha-ketoglutarate (100 mg/kg) and oxalacetate (85 mg/kg). Group V (14 animals) received the same solutions as Group III with the addition of inosine (8 mg/kg), alpha-ketoglutarate (100 mg/kg) and oxalacetate (85 mg/kg). In all groups the amount of fluid received was kept uniform.

It should be noted that these compounds had to be administered with great care since practically all of them possess vasodilator properties.

Upon completion of the administration of the various therapeutic regimens the arterial pressure was monitored for another hour and then the cannulas were removed and the skin was sutured. The dog was administered penicillin and returned to a cage. Penicillin was administered each day of survival thereafter.

The length of survival for each animal was recorded. It should be noted that the Group I animals (controls) were interspersed with the other 4 series to assure adequate control.

Results. The survival data from the five groups of animals is summarized in Table I. As was expected with this experimental design all of the Group I control animals died within 24 hr. The administration of Na allopurinol to the Group II animals afforded no protection as all of these animals died within

24 hr. The addition of the two purines, hypoxanthine and adenine, in Group III did, however, increase survival. Seven of the 10 animals were surviving after 24 hr in contrast to none in Groups I and II. At 36 hr only three were surviving of which two survived for longer than 48 hr. Only one of these survived for 2 wk. To determine if inosine and two Krebs cycle intermediates, alpha-ketoglutarate and oxalacetate, were effective in producing survival the experiments in Group IV were performed. Three of the 10 animals survived for 24 hr and were still alive at 36 hr. Two of the animals survived for at least 48 hr.

In Group V in addition to the Group III compounds in inosine was administered as as well the two Krebs cycle intermediates alpha-ketoglutarate and oxalacetate. It is significant that 10 of the 14 animals were surviving after 24 hr. Nine of these animals were surviving at 36 hr and 6 animals survived for more than 48 hr.

All of the survivors that lived for at least 48 hr were allowed to live for 2 wk before sacrifice in order to assure that these are meaningful survival rates. One of the animals in group II died before the 2 wk had passed.

It should be noted that without exception the infusion of allopurinol, the purines and the Krebs cycle intermediates invariably produced hypotensive responses in the dogs. The arterial pressures would decline quickly to 50 –60 mm Hg and remain for the time of the infusion. Great care was, therefore, required to maintain the infusion rate so that the response was minimal (approx 10 mm Hg). It was ascertained early in the experiments that too rapid an infusion of the various compounds could precipitate the death of the animals. This seemed to be an extension of the hypotensive–hypovolemic period increasing the severity of the stress.

Discussion. The term "shock" is at best a vague term used to describe a very broad and complex set of circumstances. When one adds the adjective "irreversible," the description becomes even more tenuous. However, it has been demonstrated at least in some instances, both clinical and experimental, that when certain interventions are made a number of the subjects can be "reversed" or made to survive.

Even though the dog is a questionable model for shock studies it is still widely used. As a dog goes into the state of irreversibility there is a progressive fall in systemic blood pressure and cardiac output. This is true even with over transfusion. One of the most dramatic findings is the mesenteric congestion and sloughing of the intestinal mucosa (4). It has also been demonstrated that there is reduced oxygen transport and consumption as well as release of metabolites or toxic factors and derange both the heart (5) and peripheral circulation (6). There may also be intravascular coagulation (7). These factors all contribute to the reduced oxygen transport and unexplained metabolic derangements mentioned above.

The prolonged hypoxia caused by the circulatory insufficiency led Jones, Crowell and Smith (1), Crowell, Jones and Smith (2) to hypothesize a defect in a metabolic system which obtains energy mainly from oxidative phosphorylation and provides this energy for the various functions of the cells. This system is composed of the adenine high-energy phosphate compounds such as ATP and ADP. If there is a lack of oxygen ATP formation would be limited so that there was less production than utilization. Also, as ATP and ADP are degraded, the intermediates adenosine and inosine, etc., appear and diffuse from the cell to be converted to uric acid.

There are normally resynthesis or salvage pathways for hypoxanthine and higher compounds so they can be reconverted to ATP. Jones, Crowell and Smith (1) showed that uric acid excretion is markedly elevated and this is presumably due to the conversion of purine bases which are, therefore, lost. If the purine bases are greatly depleted, then the return of oxygen presumably would not benefit the cell. Crowell, Jones and Smith (2) proposed preventing the loss of the purine base by blocking xanthine oxidase with allopurinol thus allowing hypoxanthine to be accumulated so it could be converted to ATP.

In the present experiments we found as did Crowell, Jones and Smith (2) that allopur-

inol alone did not prevent the onset of irreversible shock. We found that the addition of hypoxanthine and another purine adenine to the therapeutic regime had a minimal effect on survival. Only 20% of the animals survived for 48 hr and only one of these survived indefinitely. This is a significantly lower survival rate than found by Crowell, Jones and Smith (2) (4 of 10) when the purine base (hypoxanthine) is added. However, more recently Crowell (8) has reported 80% survival at 24 hr using this therapeutic regimen with the addition of 0.05 mg ouabain. He states that some animals die after the first day. However, no meaningful survival statistics are presented (8).

The question then was asked if other compounds that might be expected to stimulate high-energy phosphate production might be effective. We therefore, tested the effect of a combination of compounds consisting of the purine inosine, and the two Krebs cycle intermediates, alpha-ketoglutarate and oxalacetate. This regimen indicated minimal effectiveness with only 2 of 10 surviving for 48 hr or longer. Chick *et al.* (9) have demonstrated in rabbits 69% survival using the two Krebs cycle intermediates. The dosage of the Krebs cycle intermediates in their rabbits and our dogs were the same when compared on a weight basis. The difference between their data and ours could be due to the species used. However, when these compounds were combined with the allopurinol, hypoxanthine and adenine, there was a significant survival rate of 71% at 24 hr and 43% at 48 hr. These animals survived until sacrificed 2 wk later. These data would indicate that the combination of preventing the loss of purine base and the administration of other compounds that might be expected to stimulate high-energy phosphate production has definite but limited effectiveness. The metabolic derangement does not seem to be adequately corrected by this treatment.

We have tried other shock models such as altering the percentage of the blood taken from the reservoir before reinfusion as well as altering the hypotensive level. We have also tried giving the compounds a second time to the animals surviving at 24 hr and have not been able to increase long-term survival.

There is also the question of how many and how much of these compounds actually gain entry to the cells because if this type of therapy is to be effective there must be intracellular access. Since many of these compounds normally cannot pass through the cell membrane there is the possibility that the membranes might have increased permeability to these compounds as one of the derangements consequent to the hypoxic state in shock thus increasing their effectiveness.

Another aspect of this problem that must be considered is that most all of these compounds are reasonably potent vasodilators (10, 11). This includes the drug allopurinol as shown by Ellis (12). Since it is necessary to regulate the rate of infusion of these compounds by monitoring the arterial pressure so that it is minimally affected, the survival rate could be enhanced simply by eliminating the ischemia and, therefore, the hypoxia. It is possible to say the greater the number of vasodilator compounds administered and the longer time required to administer them the greater are the chances for survival. This seems to be a mode of action for such agents as phenoxybenzamine for enhancing survival (13). This is shown by Groups III and IV where only three compounds were administered and survival was only 20% in each case. In Group V where six compounds were administered the survival rate was 43% or about doubled. The infusions required 2 to 4 hr depending on the response of the dog's blood pressure and the amount of solution to be administered. It would appear that the important mode of action of these drugs could be vasodilator thereby increasing blood flow and eliminating or at least reducing the hypoxia.

Summary. The xanthine oxidase inhibitor allopurinol alone or in combination with hypoxanthine and adenine exerted little or no protection against the onset of irreversible shock. However when allopurinol is combined with the above two compounds in addition to inosine, alpha-ketoglutarate and oxalacetate 48 hr survival was increased to 43%. This may be due to prevention of loss of purine bases as well as supply of compounds

to stimulate high-energy phosphate production. The effects may also be due to the vasodilator properties of the compounds increasing blood flow and thereby reducing the hypoxic effects.

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