Effect of allopurinol on hemorrhagic shock

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CROWELL, JACK, W., CARL E. JONES, AND ELVIN E. SMITH. Effect of allopurinol on hemorrhagic shock. Am. J. Physiol. 216(4): 744-746. 1969.—Five groups of ten dogs each were hemorrhaged until their arterial pressure was 30 mm Hg, and this arterial level maintained until 20% of the shed blood had returned to the animal. The remaining blood was then reinfused. Groups I and II were used to determine the effect of pretreatment with allopurinol (Zyloprim), which blocks the action of xanthine oxidase thus preventing the irreversible loss of functional purine base. Group II, pretreated with allopurinol, showed a survival rate 6 times greater than group I, the control group, despite the fact that the treated group remained hypotensive 1.85 times longer than the control group. Groups III, IV, and V were used to determine the effect of treating "irreversible" shock. Group III was given allopurinol only after shock had been produced and it was of no benefit to the animals. Group IV was given hypoxanthine only and it also was of no benefit to the animals. However, group V, given hypoxanthine plus allopurinol to prevent the catabolism of hypoxanthine showed a significant survival rate. These results support the concept that irreversibility in hemorrhagic shock is related to loss of purine base for restoration of cellular ATP.

hemorrhage; purine metabolism; hypoxia; oxygen debt

Studies of the circulatory aspects of irreversible hemorrhagic shock eventually lead one to the metabolic system, perhaps by the chain of events as follows (4-7). Hemorrhage causes changes in many circulatory parameters, and the most important appears to be the decrease in cardiac output which results in a decrease in oxygen transport. Hypoxia per se is not the cause of shock or irreversible shock because animals can be hypoxic for a time without developing either shock or irreversible shock, rather some evidence indicates that it is the quantitative lack of oxygen, or an oxygen debt, which furnishes the basic stimulus for producing irreversible shock (8).

The oxygen debt is the stimulus to the metabolic system. After severe metabolic derangement begins to result in circulatory failure, a positive reflex is instituted whereby a decline in cardiac output results in still further metabolic injury which intensifies the circulatory failure. Recovery from the positive feedback stage has not been possible, largely because of lack of knowledge concerning the metabolic derangements produced by hypoxia.

In this paper, we make a tentative assumption as to the metabolic mechanism which causes shock to become irreversible, and furnish supportive evidence.

MATERIALS AND METHODS

Basic experiment. Adult mongrel dogs were anesthetized with 30 mg/kg pentobarbital sodium. A femoral artery and vein were surgically exposed, and a catheter placed in each. The arterial catheter was connected to a mercury manometer and a reservoir bottle. The venous catheter was used to obtain blood samples and to infuse materials. Heparin, 10 mg/kg, was injected into the venous catheter, and after time for proper mixing, the tube to the reservoir bottle was opened and the bottle adjusted so that an arterial pressure of 30 mm Hg was maintained. After the animal had taken back 20% of the shed blood, the remainder was reinfused. All animals were given 0.5 mg/kg atropine sulfate 10 min prior to reinfusion to prevent deterioration of the intestine (16), and 10 ml/kg of 5% glucose solution following reinfusion to insure a source of energy. Oxygen consumption was recorded continually by the device of Guyton (11). Samples of blood (2 ml) were taken at 30-min intervals for analysis of uric acid content by the colorimetric method of Benedict (17). The time lapse between initial hemorrhage and reversal of blood flow from the bottle was recorded as well as the total period of hypotension.

Five groups of 10 dogs each were used; two groups (I, II) were for determining the effect of allopurinol in the pretreatment of shock and three groups (III, IV, V) to determine the effect of hypoxanthine and allopurinol in postshock treatment. Group I, a control group, was given 1 ml/kg of propylene glycol (vehicle for allopurinol) 20 min before the initial hemorrhage; group II underwent the same experiment except that 50 mg/kg of allopurinol (Zyloprim) was dissolved in the propylene glycol. Group III was given 50 mg/kg allopurinol dissolved in propylene glycol 20 min after reinfusion, group IV was given 40 mg/kg hypoxanthine by drip beginning 50 min after reinfusion plus propylene glycol as was given in group III. Group V was given allopurinol dis-
solved in propylene glycol the same as group III and hypoxanthine the same as group IV.

RESULTS

Pretreated animals. Figure 1 summarizes the results of groups I and II. The data from the 20 dogs were added together and a mean for all dogs determined so that a common ordinate could be used for all measurements. The values for each of the two groups are expressed in percent of the mean of all dogs and the actual values are also shown. The reversal time for untreated dogs averaged 47 min while that of the allopurinol treated group was 83 min. The total hypotensive time of the untreated group was 73 min whereas that of the treated group was 135 min. The oxygen debt of the untreated group was 199 ml/kg and the oxygen debt of the treated group was 285 ml/kg. Yet, despite a hypotensive time 1.85 times greater, and an oxygen debt 1.43 times greater than that of the control group, the 48-hr survival of the treated group was 6 times greater than that of the control group. 6 of 10 treated dogs lived and only 1 of 10 of the control dogs lived. The increase in uric acid for the untreated dogs averaged 0.64 mg/100 ml while that of the treated dogs averaged 0.31 mg/100 ml. The increase in uric acid of the four treated dogs which died was 0.47 mg/100 ml; the increase in uric acid of the six treated dogs which lived was 0.20 mg/100 ml.

Posttreated animals. Figure 2 shows the percentage of animals remaining alive as a function of time following reinfusion for groups III, IV, and V. The curves for groups III and IV are practically identical and at the end of 24 hr, all of the dogs given allopurinol only (group III) and all of the dogs given hypoxanthine only (group IV) were dead. In contrast, of the 10 dogs given allopurinol plus hypoxanthine (group V) 6 dogs were alive after 24 hr, but 2 died during the next 24 hr; thus, 4 were alive after 48 hr. The death of these two dogs during the second 24 hr was not expected as they had been awake, walking around in their cages, and apparently not in shock the day after experimentation.

Figure 3 shows the percent change in blood uric acid level for the posttreated animals (groups III, IV, V) as well as that of the control group (I) following reinfusion of blood. Note that the data show very little difference between the control group (I), group III given allopurinol only, and group IV given allopurinol plus hypoxanthine. Group V, given hypoxanthine, showed a dramatic sustained increase in blood uric acid.

Oxygen use and blood pressure. Figure 4 shows the oxygen usage and the mean arterial blood pressure of the allopurinol pretreated group (II) and the allopurinol plus hypoxanthine posttreated group (V). The data was divided on the basis of survival. Note that the mean blood pressure as well as the oxygen consumption of the surviving animals was higher than that of the nonsurvivors.

DISCUSSION

It is neither wise nor acceptable to speculate extensively from a given series of experiments. It is permissible to outline the general hypothesis which caused the experimentation as a guide both to the meaning of the
A CONTROL ALLOPURINOL ONLY
ALLOPURINOL + Hx

MINUTES AFTER REINFUSION

FIG. 3. This graph shows primarily that treatment with hypoxanthine is not effective because of its rapid catabolism in the body.

FIG. 4. Group in which hypoxanthine loss was prevented (II) and group in which it was replaced and its loss blocked (IV) are similar. For this graph, the 2 groups were combined and then separated on the basis of survival. The data show that those dogs in which adequate oxygenation was not restored received no benefit from the treatment given.

experiments and for future experimentation. In previous papers, we have traced the chain of events from hemorrhage to inadequate oxygen transport to a quantitative oxygen debt (4, 5, 6). Also, we have shown that the heart fails and thus furnishes a positive feedback element to oxygen transport (6, 7). These have been primarily hemodynamic events. There obviously must also be some irreversible damage to a metabolic system since restoration of proper tissue perfusion does not prevent irreversible shock. At the present time we would like to discuss a metabolic system which could be altered proportional to the oxygen debt and which could cause shock to become irreversible.

The system of interest is one 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, ATP, ADP, etc. Normally, the rate of formation of ATP in this system, $e_{in}$, is equal to the rate of utilization of ATP, $e_{out}$, and the level of ATP is normal and constant. As ATP is utilized for energy, it is degraded to ADP which then provides a stimulus for oxidative phosphorylation. To restore the level of ATP to normal, a high-energy phosphate bond is added to ADP thereby converting it to ATP. However, suppose that the rate of ATP formation is limited by lack of one of the necessary components. In this case, assuming that the rate of ATP utilization from the energy reservoir remains constant, the rate of ATP loss would be

$$\frac{dQ}{dt} = k (e_{out} - e_{in})$$  \hspace{1cm} (1)$$

where $Q$ is the ATP deficit in the reservoir system and $k$ is a constant of proportionality. Since $(e_{out} - e_{in})$ represents a deficit rate, it could be replaced by $R$, and integration of equation 1 yields the equation

$$Q = kRt$$  \hspace{1cm} (2)$$

Thus, under the conditions cited, this definite integral shows that the total decrease in ATP in the energy system is proportional to $R$, the deficit rate, times time, which, if oxygen is the limiting ingredient, becomes the factors of oxygen debt. Furthermore, if the energy compounds, ATP, ADP, etc., are further degraded, the intermediates adenosine, inosine, etc., appear and diffuse from the cell to be ultimately converted to uric acid by the liver. Thus, the amount of energy compound lost from the cell to the catabolic system and subsequently converted to uric acid would be proportional to the oxygen debt.

The cell possesses resynthesis pathways such that hypoxanthine and higher compounds can be reconverted to ATP (18); these compounds are, therefore, termed functional purine bases. However, with the conversion of hypoxanthine to xanthine to uric acid by the euzynic xanthine oxidase, the functional purine base becomes irretrievably lost since the conversion of xanthine to uric acid is irreversible (2). Therefore, whereas oxygen is the limiting factor during hypoxia, the return of sufficient oxygen would not greatly benefit the cell because it has now lost so much of its functional purine base that this is now the limiting factor and the cells die for lack of substrate for resynthesis of ATP. Translated to the conditions of hemorrhagic shock, if the oxygen debt becomes great enough, a sufficient amount of functional purine base is lost so that the animal cannot survive even though his blood is restored and oxygen transport is returned to normal. It must be pointed out at this time that the above described mechanism is well documented, for studies by Imai et al. (13) and others (1, 9) have shown that isolated tissues deprived of oxygen do lose purine intermediate compounds to the surrounding fluids but recover and reconveret them if oxygen is restored.

This reconversion of ATP is possible when oxygen is
restored, since in vitro preparations do not contain xanthine oxidase which takes this catabolic schema to completion, that is, to uric acid. As previously mentioned, once the reaction has gone to uric acid, as occurs in vivo, the functional purine bases are lost.

Theoretically, if the loss of functional purine base could be prevented, shock would not become irreversible. To prevent loss of the base, it was necessary to block the action of xanthine oxidase, thus presumably allowing the accumulation of hypoxanthine, which can be reconverted to ATP. This was accomplished by use of allopurinol which competitively inhibits the action of xanthine oxidase (10). As shown under results, the actual hypoxic stimulus to the group treated with allopurinol was greater than that of the control dogs as the total hypotensive time of the treated group was 1.85 times greater and the oxygen debt was 1.43 times greater. Thus, the action of the drug was not to alter the experiment hemodynamically. Yet, despite this greater stimulus, the survival rate was 6 times greater in the treated dogs than in the control group. Thus, both the stimulus and the survival were statistically significant, and favor the concept that loss of functional purine base is the factor whereby shock becomes irreversible.

It has been shown in previous papers and abstracts that blood uric acid levels increase sharply in the hypoxia of hemorrhagic shock (3, 14). Figure 1 shows that the dogs pretreated with allopurinol produced less uric acid than the control group. Furthermore, of the four dogs in the pretreated group that died, the uric acid concentration rose to a higher level than that of the pretreated animals that lived. Since allopurinol is a competitive inhibitor, it is quite possible for some of the hypoxanthine to be converted to uric acid provided the concentration of hypoxanthine increases to a significantly competitive level and provided the time of hypotension is long. Thus, allopurinol may not provide absolute protection. However, the dosage could be adjusted to meet the demands of the experimental conditions.

The longer hypotensive period of the treated dogs had not been expected but an explanation must be provided since this indicates a very strong preventive action which could be of therapeutic value. If the sole action of allopurinol be the prevention of loss of hypoxanthine, there would be no effect on the hypoxic loss of purine from the cells and the dogs should develop shock just as readily as the control group. However, if the mass action effect prevented loss of purine base from the cell, the cell may in turn maintain higher levels of ATP during hypoxia, and a greater hypoxic stimulus would be needed to cause shock. This question cannot be answered at this time because the cellular levels of the various compounds were not determined. However, it should be noted that dipyradoine, a purine catabolism-blocking agent which prevents destruction of adenosine, increases the ATP content of myocardium during hypoxia (12).

According to the hypothesis, “irreversible” shock could be reversed by restoring the lost energy base. In one group, hypoxanthine only was given. It had no appreciable effect on the outcome as all the dogs died. Another group was given allopurinol only, and this also had no beneficial effect. The remaining group was first given allopurinol to retard loss of base, and then given hypoxanthine to replace that which was lost and the results were far different from the other two groups. On the 1st postoperative day, 6 of the 10 dogs were alive, and 4 were alive on the 2nd day.

It is quite apparent why hypoxanthine only, in the dosage given, was not effective. The degradation system removes this compound rapidly as shown by Fig. 3 and the cells have inadequate time to convert it to ATP. Whether or not larger doses of hypoxanthine would be effective was not determined. Allopurinol alone was of no benefit because presumably so much base was already lost that the animal could not survive. Thus, the combination of restoring the lost chemical plus blocking its catabolism was effective because the hypoxanthine remained available for conversion to ATP. In fact, it has been shown that incorporation of hypoxanthine into mouse liver nucleic acids is enhanced if its catabolic destruction is prevented by allopurinol (15).

The question arises as to why all of the allopurinol pretreated and allopurinol plus hypoxanthine posttreated dogs did not survive. If we refer again to the experiments of Benson et al. (1), when oxygen was restored to the hypoxic tissue, the purine intermediates were reconverted to ATP. The treatment used in our dogs was not one which caused the regeneration of ATP, but one that allowed it to occur provided that sufficient oxygen was present. In the individual experiments, the blood pressure of many of the animals remained low after reinfusion and thus the hypoxic stimulus was not removed and reconversion quite obviously could not occur. Therefore, of equal importance in preventing death of the animal is the reestablishment of adequate perfusion for sufficient time for reconversion to occur. Figure 4 shows that the blood pressure and oxygen consumption of the survivors was indeed higher than that of the nonsurvivors.

In previous experiments we had noted that atropine given prior to reinfusion prevented intestinal damage to the animals (16), and all of these animals were given atropine for this reason. Despite this, however, most of the survivors showed bloody diarrhea to some extent. Glucose was also given to all dogs to insure the presence of a source of energy.

This hypothesis concerning irreversible shock is based on the well-documented functions of the purine metabolic system including its actions during hypoxia. Our major thesis therefore is to implicate the system as the one which causes shock to develop and to become irreversible. It would appear that shock is the result of inadequate energy transfer by this system and irreversible shock results if the base chemicals of the system are changed into metabolites which cannot be reconverted to ATP. The present evidence in which it is shown that blocking the irreversible conversion does prevent shock from becoming irreversible and blocking plus restoring
the lost chemicals "reverses" irreversible shock is strong evidence favoring the hypothesis.

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REFERENCES