Laboratory Investigations

Allopurinol administered prior to hepatic ischaemia in the rat prevents chemiluminescence following restoration of circulation

Peter J. Cohen MD

Oxygen-derived free radicals produced during reperfusion may be responsible for the disturbed pathology which follows prolonged ischaemia. Measurement of hepatic chemiluminescence (low level light emission resulting from the energy released during chemical reactions of free radicals) allowed determination of whether allopurinol could prevent formation of oxygen-derived free radicals during reperfusion of the ischaemic liver. While control animals demonstrated a burst of light emission shortly after reperfusion, the rats pretreated with allopurinol showed no evidence of chemiluminescence during either ischaemia or reperfusion. It is concluded that allopurinol may modify reperfusion-induced free radical formation and possibly ameliorate the organ damage which can follow ischaemia.

Les radicaux libres dérivés de l'oxygène générés pendant la reperfusion peuvent être responsables des lésions pathologiques qui suivent l'ischémie prolongée. La mesure de la chimioluminescence (émission de lumière de faible intensité qui accompagne la libération d'énergie pendant les réactions chimiques propres à la formation des radicaux libres) a permis de déterminer si l'allopurinol pouvait prévenir la formation de radicaux libres dérivés de l'oxygène pendant la reperfusion du foie ischémique. Alors que les contrôles ont montré un jet d'émission lumineuse immédiatement après la reperfusion, les rats prétraités à l'allopurinol n'ont pas présenté de chimioluminescence que ce soit pendant l'ischémie ou la reperfusion. On en conclut que l'allopurinol peut modifier la formation de radicaux libres dérivés de l'oxygène induite par la reperfusion et prévenir partiellement le dommage hépatique consécutif à l'ischémie.

The mechanisms by which organ damage is produced by prolonged anoxia or ischaemia are incompletely understood. Although restoration of high energy stores occurs after return of normal oxygen delivery,[1] prolonged absence of organ oxygenation produces a chain of events leading to irreversible functional failure. Demopoulos et al.[2] hypothesized that the occurrence of oxygen-derived free radical-induced lipoperoxidation during reperfusion may be responsible for this damage. Peroxidative

intermediates in the brain are detected *in vitro* after a hypoxic insult.[3] That oxygen-derived free radicals might play a causative role in the pathology of reperfusion injury following ischaemia of the heart, intestine and brain has been suggested by a number of investigators.[4,5]

It has been suggested that reperfusion following ischaemia causes free radical formation.[4] In the absence of oxygen, cells cannot maintain ionic gradients and calcium leaks into the cytoplasm. Cytoplasmic calcium activates a protease which converts xanthine dehydrogenase to xanthine oxidase. While xanthine dehydrogenase cannot transfer electrons to molecular oxygen, xanthine oxidase *can* use molecular oxygen when xanthine is oxidized to uric acid. Molecular oxygen supplied during reperfusion leads to formation of superoxide radical and hydrogen peroxide. Lipoperoxidation produced by these highly active oxygen-derived free radicals is felt to be an important cause of reperfusion-induced pathology.

During lipid peroxidation, energy is released during reaction of free radical intermediates with surrounding tissue.[6] This energy appears as low-level light emission resulting from the generation of excited species (e.g., transformation of singlet to triplet oxygen) during propagation or termination of the free radical reaction. This phenomenon has been demonstrated *in vivo* by the appearance of hepatic chemiluminescence in the enzyme-induced rat during administration chloroform.[7]

Recent *in vivo* studies have documented a brief burst of light emission during the first few minutes of reperfusion of the rat liver after 45 min of ischaemia.[8] Allupurinol inhibits the activity of xanthine oxidase.[9] Itoh *et al.* demonstrated that allupurinol administration was associated with a diminution of neurologic damage in rats following cerebral ischaemia and reperfusion.[10] Since measurement of chemiluminescence *in vivo* provides a continuous real-time index of free-radical-induced lipoperoxidation, the following studies were performed to ascertain whether allopurinol administered prior to hepatic ischaemia modifies the response during reperfusion.

Methods

The study received approval of the University of Pennsylvania's Institutional Animal Care and Use Committee without difficulty; the animals were anaesthetized during the study and sacrificed immediately thereafter. Hepatic chemiluminescence was evaluated in 12 Wistar rats weighing 180–200 g as previously described.[7] A small laparotomy was performed after the intraperitoneal administration of pentobarbital (75 mg · kg^{-1}). The animal was then placed in a light-tight box to ensure freedom from artifact resulting from exposure of the photomulti-
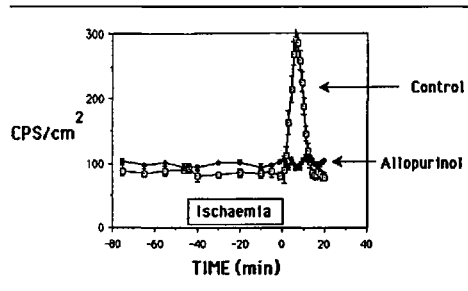

FIGURE   Mean data (±SEM) from untreated control and allopurinol-treated rats. The 45-min period of ischaemia is marked on the bottom of the graph. In control animals, light emission which had remained constant during the preocclusion and occlusion periods increased by over 300% in the first five minutes after reperfusion. Chemiluminescence returned to control values within 15 min after release of the clamp. In contrast, treated animals demonstrated no change in chemiluminescence during the period of hepatic reperfusion.

plier to outside light. Chemiluminescence was evaluated continuously with a photon counting apparatus consisting of a photomultiplier (EMI 9653) at an applied potential of 0.95 kV, an amplifier-discriminator (model 1121, Princeton Applied Research), and a frequency counter (Heathkit 1B 1100). The photomultiplier was cooled to −23° C in order to decrease dark current noise.

Six of the animals had received a normal diet of food and water; the other six had been treated identically except for the oral administration of allopurinol (200 mg · kg^{-1}) 24 hr and 1 hr before hepatic occlusion as suggested by Itoh *et al.*[10] Following a 30-min control period, the hepatic artery and portal vein were gently occluded completely obstructing circulation to the liver for 45 min. At the end of this time, hepatic blood flow was restored. Chemiluminescence was measured continuously during the control period, during occlusion, and for 20 min following reperfusion.

Results

Data from both groups are shown in the Figure; graphic representation demonstrates the contrast between the two groups most clearly. In all animals, light emission remained constant during the pre-occlusion and occlusion periods. During these two periods, values in the control and treated groups were similar. In each control animal, chemiluminescence increased over 300% within five minutes following reperfusion. Light output returned to control values within 15 min after release of the clamp. In contrast, hepatic light emission did not increase during the 20 min of observation following reperfusion in rats treated with allopurinol.

Discussion

Recently, McCord[11] proposed that "oxygen-derived free radicals (superoxide and hydroxyl) and related species (hydrogen peroxide) have well defined roles in ... post-ischaemic injury brought about by the conversion during ischaemia of the enzyme xanthine dehydrogenase ... to the radical-producing xanthine oxidase." The current investigation was designed to ascertain whether allopurinol's inhibition of xanthine oxidase might prevent the potentially harmful formation of these free radicals.

Evidence gathered in several studies provides a rationale for using chemiluminescence as an index of free radical-induced lipoperoxidation. Low level chemiluminescence accompanies the generation of electronically excited states; light emission is produced when these excited species decay to a stable state.[12] For example, light emission may occur during decay of singlet oxygen generated by either reaction of peroxy radicals or by the formation of 1,2-dioxetane derivatives.[6] Lipoperoxidation-induced chemiluminescence may be produced by infusing hydroperoxide into liver, brain and lung and by exposing liver and brain to hyperbaric oxygen.[13] A similar phenomenon is the appearance of chemiluminescence during exposure of cardiac submitochondrial particles to hydroperoxides.[14] Germane to this study is the observation that chemiluminescence occurs in brain homogenates obtained from hypoxic rats, a phenomenon considered to result from hypoxia-induced lipoperoxidation.[3]

The methodology in the current study is that used by Boveris et al.[6] They showed that free radical-induced lipoperoxidation resulting from infusion of ethyl and t-butyl hydroperoxides into the portal vein was accompanied by hepatic light emission. As much as a 30-fold increase in light output was observed; chemiluminescence decreased within 10–15 min after discontinuing the infusion. Similarly, a burst of chemiluminescence during infusion of sodium hypochlorite into the rat portal vein was described by Cohen and Chance.[7]

The present study demonstrates that continuous measurement of chemiluminescence could be used to verify the production of free radicals during reperfusion of the liver following a 45 min period of ischaemia in control animals. This burst of light emission was not observed in animals which had been pretreated with allopurinol. These data suggest that inhibition of xanthine oxidase by allopurinol may play a central role in preventing reperfusion-induced free radical formation and may ameliorate the pathology produced by their actions.

However, other explanations for these observations must be considered. Hepatic conversion of xanthine dehydrogenase to xanthine oxidase is slow, and maximum levels develop only after two hours of ischaemia.[15] Thus, the action of allopurinol on xanthine oxidase may not be the sole mechanism of action. Peterson et al.[16] demonstrated that allopurinol can act as a scavenger of superoxide radicals, a phenomenon which would certainly account for its ability to prevent perfusion-associated chemiluminescence. In this investigation, the authors also showed that allopurinol might stabilize mitochondrial membranes by facilitating the transfer of electrons from ferrous iron to ferric cytochrome c. Finally, Jaeschke et al.[17] have suggested that reperfusion-induced injury can be significantly reduced by treatment with monoclonal antibodies against neutrophils. They suggested that the injury produced by ischaemia-reperfusion might result from the neutrophils' release of reactive oxygen and proteases as well as capillary plugging by these cells. Thus, allopurinol's effect might be mediated by its action on leukocytes rather than hepatocytes. Further studies comparing the intact and in vitro perfused liver should elucidate this matter.

Regardless of the mechanism, however, allopurinol appears to prevent the deleterious effects of free radicals produced during reperfusion of the ischaemic liver. Consistent with this hypothesis is the demonstration by Itoh et al.[10] of significant diminution of neurological deficit after reperfusion of the intact rat's brain when allopurinol had been administered prior to global ischaemia. The role of allopurinol is also supported by studies of Nordstrom et al. who showed the drug's beneficial effect on protein synthesis and tissue water accumulation during reperfusion of the ischaemic liver in vivo.[18]

It is reasonable to postulate a relationship between chemiluminescence and pathology. For example, the time of onset and magnitude of hepatic chemiluminescence following administration of chloroform to barbiturate-induced rats differs from that accompanying halothane anaesthesia.[19] This parallels the differing biochemical and histologic changes in rat liver produced by the two anaesthetics.[20] However, a possible clinical role of allopurinol (e.g., in orthotopic hepatic transplantation) should not be extrapolated from the current study alone. Comparison of allopurinol's action on hepatic chemiluminescence with morphologic and functional evidence of ischaemic damage is necessary before the utility of this compound can be proven. It is of note that allopurinol is currently used in some perfusion solutions of donor livers prior to hepatic transplantation in man (Hexam J, personal communication).

In conclusion, this study demonstrated that administration of allopurinol prior to ischaemia may prevent formation of oxygen-derived free radicals during reperfusion.

Since production of these active compounds may be responsible for ischemia-induced pathology, allopurinol might be useful when ischaemia is anticipated.

References

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