Effects of Hyperbaric Oxygenation on Coronary Artery Occlusion in Pigs

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The ability of hyperbaric oxygenation to alter the course of coronary artery occlusion in man and experimental animals has not been conclusively demonstrated. The scattered reports describing the use of hyperbaric oxygenation in the treatment of myocardial infarction in human beings do not include control observations. The animal studies which have suggested that hyperbaric oxygenation may alter the course of coronary artery occlusions have little relationship to the course of events preceding and accompanying myocardial infarction in man. The dog, which has been used exclusively for these studies, is not the most appropriate animal, because the canine coronary circulation is distinctly different from that of man. Furthermore, the pattern of coronary circulation varies widely among individual dogs. The coronary occlusions have been produced either by acute ligation of a coronary artery or by embolization of the coronary circulation with microspheres. The studies have all been carried out under general anesthesia, and the duration of exposure to hyperbaric oxygenation has been two hours or less.

Because of these objections, the present study was done on the pig, an animal with a more uniform coronary circulation which resembles that of man. In addition, the coronary occlusion was induced gradually by external compression while the pigs were conscious. The animals were exposed also to hyperbaric oxygenation for periods as long as 32 hours.

Methods

A group of nonmedicated young farm pigs weighing 25 to 35 lb. were anesthetized with halothane* administered by mask. A noncuffed endotracheal tube was introduced, and the animals maintained on air and halothane by means of a Harvard respirator.† Using sterile surgical technique, a left thoracotomy was performed. After exposing the heart through a pericardiotomy, a 6-mm segment of the left anterior descending coronary artery was freed by blunt dissection. An Ameroid constrictor‡ with a 1.5 mm central lumen was then placed around the exposed vessel (fig. 1). Prior to surgery the constrictor was immersed in a 1:100 solution of benzylalkonium (Zephiran) chloride and then carefully washed in sterile saline. Placement of a constrictor of this type and lumen size will not initially interrupt blood flow. However, because of the hygroscopic nature of the Ameroid, the coronary artery will be completely occluded within 48 hours. This method has been shown to be an effective way to produce an acute myocardial infarction in the pig. Following placement of the constrictor, closure of the thoracotomy, elimination of all air from the pleural space, and initiation of spontaneous respiration, the pigs were returned to their cages. Recovery from anesthesia was prompt; most pigs were ambulatory and appeared normal three hours after operation.

An arbitrary period of 16 hours was chosen to allow recovery from the thoracotomy and for

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* Fluothane, brand of halothane, furnished through the courtesy of Ayerst Laboratories, Inc., New York, New York.
† Harvard Apparatus Company, Dover, Massachusetts.
‡ Three Point Products, Montreal, Canada.
exclusion of any animals that died as a result of surgery per se. All animals that survived this period were ambulatory and normally active with respect to eating and drinking.

The pigs were then divided randomly into two groups. Half of the animals, group A, were placed in a 2 by 4 ft animal hyperbaric chamber. The animals had freedom of movement in the chamber and were observed continuously through portholes. These animals were maintained for 32 hours at 1.25 atmospheres absolute pressure while breathing 100% oxygen flushed through the chamber at a constant rate of 10 liters per minute. The bottom of the chamber under the animal platform was covered with water to moisten the oxygen inside the tank. The temperature in the chamber was approximately that of the room (21 to 24°C). The partial pressure of oxygen (pO₂) in the chamber was greater than 800 mm Hg by measurement with an IL-113 blood and gas analyzer.* By calculation the pO₂ within the chamber was approximately 950 mm Hg.

The remainder of the pigs, group B, served as controls and were kept outside the chamber in their cages while breathing air at normal atmospheric pressure. Two to four pigs were operated each time the experiment was undertaken, following which, one or two pigs were kept in the hyperbaric chamber (group A) while one or two remained in their cages as controls (group B). Except for placement in the hyperbaric chamber, the pigs in both groups were treated similarly. The study was terminated when there were 12 pigs in each group.

All animals were observed continuously from the 16th to the 48th hour after placement of the Ameroid constrictor. Animals remaining alive in the hyperbaric chamber for 32 hours were returned to their cages. All animals surviving for 72 hours after placement of the constrictor were sacrificed with an overdose of pentobarbital. Complete gross and microscopic pathological examinations of the heart and lungs were done on all animals in both groups. Hematoxylin and eosin stain was used for all microscopically examined tissue sections. Succinic acid dehydrogenase stains were used for early detection of myocardial infarction. The pathological changes
in myocardial tissue taken from the anterior wall and septum were graded as follows (fig. 2):
Grade 0: no pathological findings;
Grade 1: minimal, spotty areas of necrosis in the subendocardium only;
Grade II: scattered subendocardial infarction;
Grade III: uniform subendocardial infarction;
Grade IV: large transmural infarction.
Several group B animals were monitored throughout the study with a transistorized electrocardiographic telemeter. This type of monitoring device could not be used on the pigs in group A because electrocardiographic signals could not be adequately recorded outside the metal chamber. The decision to allow freedom of movement to the pigs in the chamber prevented the attachment of electrocardiographic cables to the animals. This also prevented measurement of blood gases and blood pressures.

Results
A total of 67 pigs had constrictors placed on the left anterior descending coronary artery. Of these, 24 survived the arbitrarily chosen 16-hour postoperative period. Five pigs died within one hour after the operation and the remainder died at varying intervals up to 16 hours after recovering from anesthesia. Although this high mortality within the first 16 hours may have been due, in part, to postoperative complications, the most likely cause was the high mortality associated with occlusion of the left anterior descending coronary artery in the pig. All animals surviving more than 16 hours were autopsied within the first two hours after death, and each Ameroid constrictor lumen was found small enough to occlude completely the left anterior descending coronary artery.

Pigs in group A treated by hyperoxia lived for periods of 24 to 72 hours after surgery. The mean survival time for the group was 46.8 hours. Two animals lived 72 hours and were sacrificed. Pigs in group B (nontreated controls) lived 16.5 to 31 hours with a mean survival time of 21.3 hours. No pigs in group
B survived for the 72 hour maximum (fig. 3). The majority of pigs in group A had significant myocardial infarction as shown with succinic acid and dehydrogenase stains, whereas only two animals in group B had evidence of myocardial damage (fig. 4). Only the three longest survivors in group A (the two sacrificed at 72 hours and the one dying at 60 hours postconstrictor placement) had evident myocardial infarctions by hematoxylin and eosin stain, denoting damage of several hours duration. No animals in group B had grade III or IV infarcts while eight pigs in group A developed lesions of this degree. In general, the longer the animals survived, the larger was the myocardial infarction found at autopsy (fig. 5).

Death occurred suddenly without forewarning in both groups of animals. Analysis of telemetered electrocardiographic records in group B indicated that death was due to ventricular fibrillation (fig. 6). Since the animals in group A died in the same fashion as those in group B, it is presumed they also died because of ventricular fibrillation.

No animals in the study presented pulmonary changes suggestive of oxygen intoxication.20,21 One animal in group A, surviving 60 hours, had a grade IV myocardial infarction and changes compatible with mild congestive heart failure. Several animals in both groups had minor atelectatic changes in the lungs. The animal in group A that survived only 24 hours was found to have had complete atelectasis of the left lung. In retrospect, this finding was thought to be a late complication of surgery and may well account for the early death of this animal despite treatment by hyperbaric oxygenation. The next shortest survival time in the treated group was 32.5 hours.

The observed results could not be correlated with sex, weight or age of the pigs. The animals were usually five to six weeks old. The average weights in both study groups were approximately the same (group A: 29.4; group B: 30.0 lb.). When pigs less than 20 lb. or greater than 40 lb. were used during a preliminary study under normal atmospheric conditions, the survival times were longer and shorter, respectively. This result was presumably due to the disparity in size of coronary vessels with respect to the lumen of the constrictor in the larger and smaller animals.

In a separate group of five animals a "sham procedure" was used, i.e., the same operation as in the experimental groups, including dissection of the left anterior descending coronary artery, but excluding application of the constrictor. These animals were placed in the chamber under the same atmospheric conditions as the group A (hyperoxic) animals. All animals in this group survived 72 hours and, when sacrificed, exhibited no pathological evidence of myocardial infarction or of pulmonary oxygen intoxication. In addition, a separate group of three pigs was placed in the hyperbaric chamber rather than in their cages 16 hours after application of the Ameroid constrictor and were allowed to breathe air at normal atmospheric pressure. These animals had essentially the same survival times as animals in group B (control animals). Ameroid constrictors immersed in saline closed in the same amount of time.

**Figure 4**

Severity of heart lesions in both groups. Note low incidence of myocardial infarction in control animals.
when tested in the chamber at 1.25 atmospheres absolute pressure with 100% oxygen as they did when exposed to normal atmospheric pressure and air. These additional studies indicate that the operative procedure itself, the confinement associated with treatment in the hyperbaric chamber, and possible differences in closure time of the Ameroid constrictors due to hyperoxic exposure cannot account for the prolonged survival observed in the animals with hyperbaric oxygenation.

**Discussion**

The rationale for the use of hyperbaric oxygenation in the treatment of myocardial infarction has been that coronary arterial blood can contain enough physically dissolved oxygen to limit significantly the areas of tissue ischemia surrounding a myocardial infarction. It has been suggested that elimination or diminution of these ischemic areas may eliminate the foci of ventricular arrhythmias which precipitate sudden death. It is well known that fatal ventricular arrhythmias can arise not only from a very localized area of acutely altered myocardium but can occur even in the absence of demonstrable necrotic muscle tissue.

It is interesting that in the group of control animals telemetered in our study, death did occur suddenly and was due to the onset of ventricular fibrillation or of ventricular tachycardia followed immediately by ventricular fibrillation. Other studies on arrhythmias in animals after acute ligation of a coronary artery and the few observations in human beings electrocardiographically monitored following acute myocardial infarction have shown that ventricular tachycardia and/or ventricular fibrillation are commonly the terminal event. Certain studies have demonstrated that some degree of protection from...
Electrocardiographic changes following placement of Ameroid constrictor. Electrocardiographic lead V1 was used in all animals monitored. This representative electrocardiogram shows early T-wave changes at 15 hours which progressed to severe changes two hours later. One premature ventricular contraction not shown occurred one minute prior to the terminal unremitting arrhythmia.

sudden ventricular fibrillation can be afforded dogs with acute myocardial infarction by exposing them to short periods of hyperbaric oxygenation during or soon after acute ligation of a coronary artery.

The present study was designed to determine the effects of longer treatment with hyperbaric oxygenation in a form of experimental myocardial infarction which resembles more closely the clinical situation occurring in man. The pig was chosen for these experiments because this species has a coronary circulation similar to that of man. However, it was recognized that the coronary arteries of these young pigs, unlike those of men, were free of arteriosclerotic lesions. The myocardial infarction was induced by a special constrictor which produced a progressive, slow occlusion of a coronary artery over a 48-hour period. This technique allowed the infarction to take place while the animals were no longer under anesthesia and were normally active. Furthermore, this form of infarction seemed to simulate more closely that which might be occurring in human beings who develop coronary insufficiency followed by frank myocardial infarction.

Although occlusion of the right or left circumflex coronary artery in the pig is associated with a lower mortality,15,16 the choice of the more lethal left anterior descending coronary artery preparation provided two distinct advantages. Any benefit derived from hyperbaric oxygenation would be more obvious in a small series of animals. The survival time could be measured in hours rather than days, which allowed observers to be constantly at hand throughout the study.

The exact schedule for the length of hyperbaric exposure, the gas mixture used, and the atmospheric pressure at which to work were evaluated in our laboratory during a preliminary study. A major problem was the determination of an adequate exposure time which would also eliminate the risk of oxygen intoxication to the lungs.* Furthermore, it was desirable to determine if only moderate grades of hyperbaric oxygenation might be protective, because this would greatly simplify treatment schedules by eliminating the need for long decompression schedules. In essence, we sought to find the simplest treatment regimen which would provide the greatest safety and

*It was observed that the pig does not tolerate prolonged hyperbaric oxygenation even at pressures as low as 2 atmospheres absolute. Those exposed to this regimen succumbed in 12 to 15 hours. Pigs placed in a hyperbaric chamber at 2 atmospheres absolute pressure and allowed to breathe 100% oxygen for 2 hours followed by a half-hour period of air inhalation died in approximately 20 hours. None were observed to have had seizures. All animals showed a severe degree of pulmonary oxygen intoxication on pathological examination.

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the fewest problems in a decompression schedule. For these reasons we chose the relatively moderate hyperbaric oxygenation schedule of 1.25 atmospheres absolute which is calculated to give an arterial pO₂ of 950 mm Hg. Pigs could be exposed to oxygen under these conditions for at least 32 hours without detrimental effects.

The present study amply demonstrates that moderate hyperbaric oxygenation can decrease significantly the mortality associated with experimentally induced myocardial infarction in pigs. The striking survival differences in the two groups were highly significant (P < 0.001), and were presumably due to the hyperbaric oxygenation. With the exception of only one animal, all pigs treated with hyperbaric oxygenation outlived those pigs which were not treated in such a manner. The only exception to this observation was an animal that had complete atelectasis and infarction of one lung at autopsy, but no histochemical evidence of myocardial infarction. The shortened survival time of this animal may well have been a function not only of the coronary occlusion, but also of the respiratory insufficiency associated with the functional loss of one lung.

The pathological examinations of the hearts demonstrated that myocardial infarctions could not be found in 10 of the 12 control animals, all of which died early after placement of the Ameroid constrictor. However, in 9 of the 11 animals that were treated with hyperbaric oxygenation and lived for a longer period of time, varying degrees of myocardial infarction were discovered. Indeed, the most severe forms of myocardial infarction noted pathologically were in those animals which lived longest. This was presumably related to the finding that the moderate hyperbaric oxygenation prolonged the life of the animal and permitted the pathological process of infarction to evolve and become apparent histologically. The control animals, on the other hand, died apparently before actual infarction could be identified histologically or histochemically.

Summary
To evaluate more precisely the possible protective effect of hyperbaric oxygenation in experimental myocardial infarction, gradual occlusion of a coronary artery was produced in the pig. Ameroid constrictors designed to produce complete occlusion within 48 hours were placed on the left anterior descending coronary artery of 24 young farm pigs. Of these, 12 were placed in a hyperbaric chamber at 1.25 atmospheres absolute and allowed to breathe 100% oxygen for a maximum of 32 hours. For control studies 12 other pigs were handled in a similar manner outside the chamber while breathing air at normal atmospheric pressure. Pigs treated with hyperbaric oxygenation outlived the untreated pigs. Pathological and histochemical studies of the hearts revealed that pigs treated with hyperbaric oxygenation had a high incidence of myocardial infarction, whereas only two pigs in the untreated group showed any evidence of myocardial damage. These findings suggest that moderate but prolonged hyperbaric oxygenation may lengthen life during and after coronary occlusion. This prolongation of life may permit the development of pathologically detectable myocardial infarctions.

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