Experimental Myocardial Infarction

Induction of Coronary Thrombosis in the Intact Closed-Chest Dog

By Andres E. Salazar, M.D., F.A.C.P.

THE DIFFICULTY of inducing consistent, reproducible, segmental arterial thrombosis in the experimental animal has been highlighted in recent literature and constitutes one of the stumbling blocks in the study of occlusive arterial disease. As pointed out by J. R. O'Brien in his exhaustive review of the subject, "the immediate precipitating stimulus necessary for the formation of a thrombus in an apparently healthy vessel remains completely unknown."

Our interest in the phenomena associated with acute myocardial infarction led us to search for a method of producing localized segmental occlusion in a major coronary branch in the experimental animal. It was our purpose to eliminate, if possible, the surgical trauma and the hemodynamic and local vascular alterations incident to an open-chest preparation and, at the same time, approximate the usual sites of occlusion as observed clinically and pathologically.

Numerous methods have been employed successfully to produce myocardial necrosis in the intact animal. Embolization with a variety of agents, such as graded microspheres, oil, air, vegetable spores, and suspensions of particulate matter, will lead to tissue necrosis by vascular occlusion at the arteriolar level. Sclerosing solutions, caustics, and other necrotizing agents including radiopaque media when injected into the coronary arteries will also produce clinical and electrocardiographic evidence of myocardial damage and histological changes of interstitial hemorrhage and tissue necrosis. The distribution of these changes, however, is patchy and largely unpredictable. None of these methods satisfied our criteria for duplicating the physiological insult of an area involvement characteristic of main trunk or major branch thrombosis. The focal disseminated nature of the vascular occlusion in the finer arborizations of the coronary tree precludes an adequate evaluation of the reparative process and of collateral channel formation from neighboring vessels.

The work of Sawyer et al. in 1953 offered a novel line of approach. Their study of the electrical potentials of the walls of intact and injured blood vessels and the relation of these potentials to intravascular thrombosis led them to conclude that the essential prerequisite for in vivo thrombus formation was probably a loss of, or a reduction in, the degree of the normal electrical negativity of the vascular endothelium in relation to the adventitia. Based on these assumptions, we devised a modification to the West coronary catheter to permit the passage of an electrode into the arterial lumen (fig. 1).

Methods

Mongrel dogs of both sexes, between 10 and 20 Kg. in weight, were used in all experiments. After light anesthesia with intravenous pentobarbital sodium, a modified West catheter was passed from a carotid artery into the left coronary artery and advanced beyond its bifurcation into either the anterior descending or the circumflex branch. Under fluoroscopic guidance through an image intensifier, a Teflon-coated stainless-steel electrode* exposed at its tip for about 3 mm. was advanced through the catheter to the desired location in the lumen of a major trunk. The position of the electrode was then confirmed.

From the Departments of Pharmacology, Physiology, and Medicine, Temple University School of Medicine, Philadelphia, Pennsylvania.

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by the injection of a small amount of Renografin and a cinefluorographic recording of the arterial pattern made at the same time. Because of the undesirable effects of the selective intracoronary injection of radiopaque materials, the minimum amount required to obtain visualization was used, usually 1 or 2 cc. The intraluminal electrode was connected to the positive side of a circuit, which was completed by a negative electrode on the chest wall. Continuous monitoring of the electrocardiogram and femoral blood pressure was maintained throughout the experiment with a multichannel recorder as described by Oppenheimer et al. The cinefluorographic technique was similar to that employed by these authors. A direct current from a three-volt dry-cell battery was then introduced into the circuit and the intensity regulated by means of a 100,000-ohm Helipot potentiometer. Except for a baseline shift, there was no electrocardiographic alteration when the circuit was closed. The intensity of the current was then gradually increased, as registered by a Triplet microammeter, to between 100 and 900 microamperes or until premature ventricular systeroles appeared. Electrocardiographic runs of the six standard limb leads and one intracoronary lead were taken at intervals of 5 to 10 minutes or as deemed necessary. The current flow was continued until definite electrocardiographic evidence of myocardial ischemia was noted, at which time the presence of occlusion was confirmed by coronary arteriogram. The electrode and catheter were then removed and the wounds closed. Penicillin was administered intramuscularly, and the animal returned to its cage without further medication. All hearts were examined either at the time of spontaneous death or when the animals were sacrificed at intervals varying between eight hours and 16 days postinfarction.

**Results**

In all, 23 experiments have been conducted with no mortality attributable to the operative procedure per se. In all animals major branch
occlusion was induced in periods ranging between 18 and 93 minutes of current flow. The time required for total occlusion seemed to be related to the intensity of the current employed and to the diameter of the vessel. In 17 animals the anterior descending branch of the left coronary artery was occluded 1 to 3 cm. from its origin. In the remaining six animals the left circumflex branch was thrombosed at points 0.5 to 4 cm. beyond the bifurcation. Postmortem dissection of the obstructed vessel in some animals showed that the thrombus propagated for a short distance proximal to, and for longer distances distal to, the area of electrode contact and often extended into branches originating from the main vessel (fig. 2). Smaller vessels in the vicinity of the electrode and presumably within its electrical field also showed thrombus formation in their lumina. Myocardial infarctions of varying sizes and types, depending on the competence of the collateral circulation, were uniformly present in the area of distribution of the involved artery (figs. 3, 4, and 5). Microscopic examination of these areas showed the changes characteristic of myocardial infarction in the corresponding stage of evolution. When high-intensity current was used (between 600 and 900 microamperes), serial sections of the thrombosed vessel revealed marked intimal and subintimal injury at the point of electrode contact, often extending to the muscularis. The thrombus was firmly attached to the vessel wall at this point and extended proximally and distally beyond the area of injury. Histologically it was indistinguishable from spontaneous intravascular thrombi and presented the expected stage of organization, in one instance early recanalization being apparent. The area of electrode vascular injury usually was accompanied by a more or less intense inflammatory reaction and perivascular cellular infiltration that evolved to fibroblastic replacement within a few days (figs. 6, 7, and 8). Currents less than 200 microamperes produced only minimal or no microscopic alterations in the intima and subintima, but the thrombus showed identical histological features and a similar firm attachment at the area of electrode contact. With reversal of the polarity of the circuit, thrombosis failed to occur unless high-intensity currents, with the production of an electrical burn of the vessel wall, were employed.
Obstruction of the anterior descending branch of the left coronary artery. The patent circumflex branch is filled by reflux of dye injected into the occluded artery. Reproduced from a 16-mm. cinefluorographic film frame taken 30 minutes after induced thrombosis.

In two animals the catheter and electrode were introduced into the left circumflex branch and allowed to remain in situ with no current flow for two and three hours respectively. There were apparently no significant alterations of the circulatory dynamics. The vessels maintained a normal caliber as determined by arteriograms, and the electrocardiogram remained unchanged. The catheter and electrode were then withdrawn and passed into the left anterior descending branch where electrothrombosis was induced in the usual manner. At postmortem examination, the first site in the circumflex branch was normal grossly and microscopically. The mechanical presence of the metallic foreign body within the arterial lumen apparently does not constitute a stimulus of sufficient intensity to cause a significant degree of vasospasm or to induce clot formation and persistence under the conditions of our experiments.

Unipolar electrocardiographic tracings using the intraluminal electrode as an epicardial lead showed injury currents and signs of myocardial ischemia with marked ST-T changes long before they became apparent in the standard and unipolar limb leads. The latter usually appeared 10 to 30 minutes later and as a rule progressed rapidly to the classic pattern of myocardial infarction. Arteriograms done at the time of the initial appearance of epicardial-lead abnormality usually showed a filling defect at the electrode site and a delayed emptying time of the vessel distal to it.

Even after the electrocardiographic evidence of infarction was well established and the electrode withdrawn, some degree of patency of the vessel was often demonstrable on arteriograms performed immediately afterward, but occlusion became complete within a short time, presumably by thrombus growth into the space vacated by the electrode.

Discussion

The advantages of the use of a closed-chest animal in the study of certain parameters of the evolution of a myocardial infarction can not be overemphasized. Thoracotomy introduces immediate and profound hemodynamic alterations. Pericardiotomy with exposure of
the heart is well known to influence the mechanisms of collateral channel formation and of myocardial revascularization significantly. Acute surgical ligation of a major coronary vessel carries a high incidence of morbidity and mortality, with the frequent development of ventricular fibrillation. The two-stage occlusion or the slow thrombosis by the implantation of aluminum-magnesium alloy needles, as reported by Swedish investigators, although less insulting to the electrical balance of the conduction mechanism and accompanied by a considerably lower incidence of ventricular fibrillation, still involves the disadvantages of pericardiotomy. The intraluminal-electrode method provides for a gradual occlusion that can be controlled to some extent by regulation of current intensity. The rate of thrombus growth to total occlusion can be prolonged to several hours, thus approximating the sequence of events that probably takes place in clinical coronary thrombosis.

It is an accepted fact that normal blood in contact with injured endothelium will lead to clot formation in vivo. The degree and nature of endothelial injury and the interaction of the liberated "tissue factors" with the blood substrates and activator systems will determine whether or not clot formation will progress. Beyond the mechanism of thrombus formation arises the equally important question of clot persistence. Enough evidence exists to substantiate the view that, unless the rate of thrombogenesis exceeds that of concomitant active thrombolysis, clot maturation and establishment will fail to occur. As observed by us, an electrical burn of the vessel wall with considerable tissue damage and periarterial inflammatory reaction will be produced when currents of relatively high intensity (more than 500 microamperes) are employed, regardless of the polarity of the intraluminal electrode. In this situation, factors in the coagulation mechanism will be brought into play and thrombogenesis will proceed as it will in any other instance of
adequate intimal injury, be it mechanical stripping, crushing, inflammation, anoxemia, or atheroma. “Injury currents” generated in the damaged tissue may participate in the initiation of the electrobiophysical chain reaction that culminates in the formation of a clot and vascular occlusion. These currents have been shown to result in a reversal of the normally negative charge of the intima and in the development of a strongly positive potential in relation to the adventitia.

In the low current range, however, when no histological alteration of the vascular endothelium is apparent and theoretically no injury currents are being generated, the “positivity” necessary for the cataphoretic capture of platelets and the initiation of thrombogenesis is provided by the electrical field surrounding the intraluminal electrode.

The complexities of the coagulation mechanism are far from clarified even in controlled in vitro experiments. The in vivo counterpart presents still greater difficulties of approach and analysis (see bibliography in O’Brien). Aware of the shortcomings of any attempt to explain the electrophysiological phenomena involved, the above method is offered pragmatically as one with a high degree of predictability and reproducibility, which we have found very useful in our work. Theoretically it is applicable to any vascular system that can be approached by catheterization and involves a low operative morbidity in the experimental animal.

Summary

Coronary thrombosis can be consistently induced in the intact closed-chest dog by the passage of a low-grade direct electrical current through a circuit that includes a positive intraluminal electrode in a major coronary branch and a negative external electrode on the chest wall.

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Andres E. Salazah

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