The Effect of Cortisone on Experimentally Produced Myocardial Infarcts

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Administration of a moderately large or large dose of cortisone to dogs having acute myocardial infarctions inhibited slightly the rate of removal of necrotic muscle fibers in these animals as compared with untreated animals. Delay was appreciable at four and six days after production of the infarct in animals receiving 2.5 mg. of cortisone per kilogram of body weight and at 4, 6, 12 and 21 days in animals receiving 10 mg. per kilogram. At all other periods up to essentially complete healing of the infarcts as defined at 60 days, no appreciable differences existed between treated and untreated animals.

With the general availability of cortisone, the study of its influence on such fundamental body reactions as inflammation, connective tissue growth, and immunologic responses has been possible. That administration of cortisone does materially alter these reactions in many animals under certain conditions is now well known.

The lack of information concerning the effect of cortisone on the healing of myocardial infarcts prompted the investigation of this problem in dogs. During the formulation of this problem, two reports on the results of investigations along these lines appeared in the literature.1, 2 The fact that strikingly different results were obtained by two groups of investigators (Johnson and associates; Chapman and associates) made the problem all the more inviting to pursue.

Johnson and associates1, 3 reported on the effects of cortisone on experimentally produced myocardial infarcts in dogs. When they gave the animals 25 to 40 mg. of cortisone daily beginning at the time of ligation of the anterior descending coronary artery, they found that the resulting myocardial infarcts were strikingly smaller than were the infarcts in the animals of the control group, which underwent the same operation but did not receive cortisone. They also found fewer adhesions in the surgical area, less postoperative morbidity, a lower mortality rate, and a marked decrease in fibroblastic proliferation in the animals treated with cortisone. Increased vascularity of the infarcts in the treated animals was also noted.

Chapman and co-workers4 gave 7 mg. of cortisone per kilogram of body weight daily to a group of dogs and ligated the anterior descending coronary artery in its proximal third. Control animals were similarly operated on but did not receive cortisone. Examination of the infarcts in 10 and 30 days disclosed no gross differences between the animals receiving cortisone and the control animals. Slightly less coagulative necrosis was noted in the infarcts in animals which had received cortisone for 30 days than was noted in the control group. No difference in the inflammatory response or in the degree of fibrosis was noted.

Subsequent to a report of our investigation published in abstract form,4 Opdyke and his associates5 reported on their attempts to duplicate the experiments of Johnson and co-workers. They found no difference in mortality, in the number of adhesions, or in the size, rate of healing or vascularity of the infarcts between the cortisone-treated and the control animals.

Methods

It was decided that this study should involve 36 dogs. Thus, whenever an animal died, another animal was operated on and given the type of treat-
ment which the original animal had been designed to have. These 36 mongrel dogs, weighing from 12 to 17 kg., were divided into three equal groups: a group receiving injections of a control solution, a group receiving cortisone intramuscularly in a dose of 2.5 mg. per kilogram of body weight daily, and a group receiving 10 mg. of cortisone per kilogram of body weight daily. In those animals receiving cortisone, administration of the drug was begun 48 hours prior to the operation to be described and was continued until death, the last injection being given within 16 hours of the time of death. The total daily dose was administered in two equal injections. The same schedule was followed in injecting animals of the control series save that in the place of the cortisone solution a solution was used which contained, except for carboxymethylcellulose, all of the agents of the suspending vehicle of "cortone" in similar concentrations.

With the animals under ether anesthesia myocardial infarcts were produced by ligating the anterior descending coronary artery. This was done at such a distance from the origin of the artery that the area of myocardial cyanosis which appeared involved the anterior surface of about the apical third of the left ventricle.

Ligation of secondary, tertiary or large anastomotic communications was done occasionally in an effort to produce the desired area of myocardial cyanosis. The average distance of the point of ligation from the origin of the anterior descending coronary artery was 3 cm.

The technic of ligation included the method described by Harris,4 which involves constricting, for a period of time, the coronary artery to be ligated in an attempt to reduce the incidence of fatal arrhythmia which may occur at the time of complete interruption of the artery. At the selected point the artery was isolated and then constricted by ligating it over a 20 gage needle. The needle was immediately extricated with a resultant reduction in the diameter of the lumen of the vessel to that of the outside diameter of the needle. This constriction was maintained for an average of 22 minutes in 35 of the 36 experiments. In one animal, the artery was ligated abruptly. During the period of constriction a segment of artery below the ligature, varying in length between 0.8 cm. and 2.5 cm. (average, 1.4 cm.), was isolated. Following the period of constriction this isolated segment was ligated and excised so that any communicating channels which might bypass a single ligature were disrupted.

The pericardium was then closed after 100,000 units of crystalline penicillin had been instilled into the pleural and pericardial spaces. Each animal was given 300,000 units of depot penicillin intramuscularly at the time of operation and daily for two days thereafter except for those animals in which the experiment was terminated in two days. In this case the injection of the second day was omitted.

At 2, 4, 6, 12, 21 and 60 days after the operation at which myocardial infarction was produced, two animals from each of the three treatment groups were killed either by an overdose of ether or by exsanguination while the animals were anesthetized, and the infarcts were studied in the manner to be described. Necropsy was performed immediately after death.

Sections of the hearts were stained variously, depending on the age of the infarct. Sections from two-day-old infarcts were stained only with hematoxylin and eosin. Those from four-day-old infarcts were stained with hematoxylin and eosin, Mallory-Heidenhain and Mallory's phosphotungstic acid hematoxylin stains. Sections from the 6 and 12-day-old infarcts were stained, in addition, with van Gieson's connective tissue stain. Sections from the 21 and 60-day-old infarcts were stained with the same stains except that van Gieson's stain was replaced by Verhoeff's elastic tissue stain with a van Gieson counterstain.

The sections from the infarcts in animals of the control group were studied first. The features of the healing processes at the different age intervals were noted. Then the identifying labels on all slides from the infarcts of animals having received cortisone were covered so that the microscopic examination of the sections was made without knowledge as to the age of the particular infarct being studied or the dosage of cortisone which the animal had received. In this part of the examination an attempt was made to estimate the age of the infarcts as a means of judging whether the rate of healing was different or similar to that of the infarcts in the control group. Then, all slides from the infarcts of the six animals in each age group including the controls were studied as unknowns and the healing processes were compared.

Results

The mortality rate in this series was 14.3 per cent. Of the six animals that died, five had received cortisone and one was a control. Of the five deaths occurring during operation, four resulted from ventricular fibrillation and one from ventricular standstill. One death occurred six hours after operation. Of the 42 animals operated on, 29 had been treated with cortisone and 13 were controls.

All of the surviving animals tolerated the surgical procedure remarkably well, and no consistent difference in postoperative recovery rate was noted between the cortisone-treated and the control animals. One animal, in the group receiving the larger
Fig. 1. A two-day-old infarct in one of the animals receiving 10 mg. cortisone per Kg. of body weight daily. The heart has been sliced transversely parallel to the base. Each slice is approximately 1 cm. thick. Infarcts of this rather large size were produced in both cortisone-treated and non-cortisone-treated animals.

dose of cortisone for 62 days, had to undergo reoperation on two occasions because of separation of the deeper layers of the thoracic incision. This animal received penicillin daily over a longer period of time than did the other animals comprising this study. The incision eventually healed satisfactorily while the animal continued to receive cortisone.

As mentioned in the section devoted to the description of methods, the microscopic sections from the infarcts were studied as unknowns by two methods.

First, the labels on the slides from the infarcts of all animals having received cortisone were covered, and the slides were studied as unknowns. These were properly identified as to age, with the following exceptions: The six-day-old infarcts of the animals receiving the larger doses of cortisone were incorrectly interpreted as being infarcts of four days' duration. Also, the four-day-old infarcts, both of the group with the smaller and of the group with the larger doses of cortisone, while interpreted as four-day-old infarcts, seemed to have less evidence of removal of necrotic muscle than had the 4-day-old infarcts of the control animals.

Secondly, the infarcts from the six hearts in each age group were compared grossly and microscopically without knowledge to which treatment group each specimen belonged. Later the key was exposed, allowing comparison to be made. The following results were obtained from this second method of study.

Two-day-old Infarcts. There was no difference in the amount of fibrinous exudate on the pericardial surfaces, in the appearance of the necrotic muscle, in the degree of polymorphonuclear infiltration, or in the number of extravasated erythrocytes among the infarcts of the cortisone-treated as compared to the control group (fig. 1). The very minimal degree of resorption of necrotic fibers which was observed in the control group was not seen in either of the treated series.

Four-day-old Infarcts. No gross differences between the infarcts of animals receiving cortisone and those not receiving cortisone were noted. Microscopically, resorption of necrotic muscle in the hearts of all animals receiving cortisone was somewhat less than in the controls (fig. 2a and b). The dose of cortisone apparently did not influence the degree of inhibition of resorption. There was no appreciable difference in the degree of polymorphonuclear infiltration or in the number of mitotic figures among fibroblasts in the cortisone-treated as compared with the control subjects.

Six-day-old Infarcts. Grossly, the narrow gelatinous zone, the zone of resorption immediately peripheral to the necrotic mass of muscle, did not appear to be as wide in the infarcts of dogs receiving 10 mg. of cortisone per kilogram of body weight daily as in the control group. No difference was noted in this respect between the control group and the group receiving the smaller dose of cortisone.

This was confirmed microscopically by a narrower zone from which muscle fibers had been removed in the animals receiving 10 mg. of cortisone per kilogram of body weight daily (fig. 2c and d). The sections from the group receiving 2.5 mg. of cortisone per kilogram of body weight daily showed a similar but less evident deficiency in removal of necrotic muscle fibers as compared with the controls. With the aid of special stains it was established that there was slightly less collagen formation in the...
Fig. 2. Comparison of the degree of healing in four and six-day-old infarcts in cortisone-treated and non-cortisone-receiving animals. (a) and (b) Four-day-old infarct. Zone of resorption next to surviving myocardium which appears on left side of the illustrations. (a) Control. Note that resorption of necrotic muscle has occurred in the infarcted area (hematoxylin and eosin; X80). (b) 10 mg. cortisone per kilogram. Compared to (a), there has been less resorption of necrotic muscle (hematoxylin and eosin; X80). (c) and (d) Zone of resorption and fibroplasia in six-day-old infarcts. (c) Control. Demonstrates the almost complete absence of necrotic muscle and the condensation of stroma in the most active area of repair (hematoxylin and eosin; X275). (d) 10 mg. cortisone per kilogram. The area presented here corresponds to that in (c). Necrotic muscle still remains and there has been less condensation of stroma (hematoxylin and eosin; X300).
peripheral portions of the infarcts of the animals receiving the larger dose of cortisone than in the other two groups. No difference regarding collagen formation was observed between the infarcts of the control animals and of the animals receiving the smaller dose of cortisone.

Twelve-day-old Infarcts. The features of the infarcts from the animals receiving the smaller dose of cortisone were no different than those of the controls. In the animals receiving the larger dose of cortisone necrotic muscle had not been removed from as large an area as it had in the controls. Condensation of stroma and collagen formation were, however, qualitatively similar.

Twenty-one-day-old Infarcts. Grossly, in all infarcts of cortisone-treated animals, some necrotic muscle was still identifiable. No other gross differences were observed.

No microscopic differences in the nature and size of the scar were noted between the controls and the group receiving the smaller dose of cortisone, save for the presence of some unremoved infarcted muscle in the latter. Although the infarcts of the two animals receiving the larger dose of cortisone differed slightly between themselves in degree of scar formation, it was felt that in each it was slightly less as compared to that in the controls. The scar tissue present was as densely collagenized as in the controls.

Sixty-day-old Infarcts. There were no essential differences grossly or microscopically between the infarcts of cortisone-treated and control animals. The infarcts in the two control animals were smaller in size than those in the animals receiving cortisone. All infarcts were healed and in all there was much dense collagen. Elastic tissue fibers were present in all scars of this age group.

Comment
A study such as this could only be undertaken once it had been established that the healing processes in experimentally produced infarcts in dogs follow a consistent and predictable pattern with definite time relationships. The close similarity in the degree of healing observed between the two control animals of each age group and the two animals in each cortisone-treated age group established this fact and so permitted the more detailed comparisons between the cortisone-treated and the control groups.

That the healing processes in experimentally produced myocardial infarcts in dogs have a predictable relationship with age is further borne out by the fact that the pattern of healing of infarcts in the control series in this experiment correlates well with that reported in dogs by Karsner and Dwyer. Certain exceptions were observed. It was thought that most of these exceptions were attributable to the larger size of the infarcts in this study as compared to theirs. The microscopic findings in this series of infarcts compared quite well with those described by Mallory and associates in man except that healing proceeded at a considerably more rapid rate in the infarcts of the canine hearts composing this investigation.

Any attempt at standardization of the size of experimentally produced myocardial infarcts would be fraught with much disappointment. The vagaries of the arterial circulation to any area of the heart make the size of the infarct resulting from ligation of a given artery quite variable. It was believed that ligation of the anterior descending coronary artery and inclusion of more or less of the vessel or its branches as needed to produce an area of cyanosis which included the anterior apical portion of the left ventricle would come closer to producing a standard-sized infarct than routine ligation at any given level. This method was here employed.

Cortisone had no appreciable influence on the degree or type of cellular infiltration at any stage of healing. It likewise had no effect on the vascularity of infarcts which could be detected microscopically.

Cortisone in either dosage delayed the healing processes, but only at certain phases. This retardation in healing resulted from delay in the removal of necrotic muscle from the infarct. This delay in removal of infarcted fibers could not be related to reduced numbers of neutrophils or macrophages, since these cells appeared to be present in as large numbers in the cortisone-treated as in the control series.

Cortisone did not have any appreciable
effect on fibroplasia as determined by the cellularity, by the number of mitotic figures, or by collagen formation once an area had been cleared of necrotic muscle. The density of the scar tissue formed was essentially the same in treated animals as in controls.

The results of this investigation do not confirm the findings of Johnson and associates that cortisone has a marked effect on the size and healing of myocardial infarcts. They do, in essence, substantiate the observations of Chapman and co-workers. The larger dose of cortisone employed in certain of the experiments reported here probably accounted for the greater disturbance in healing noted in these experiments than in those described by Chapman's group.

SUMMARY AND CONCLUSIONS

In this investigation, 36 mongrel dogs were divided into three equal groups; a group receiving injections of a control solution, a group receiving 2.5 mg. of cortisone, and a group receiving 10 mg. of cortisone intramuscularly per kilogram of body weight daily. At 2, 4, 6, 12, 21 and 60 days after the operation at which myocardial infarction was produced by ligating the anterior descending coronary artery, two animals from each group were killed and the infarcts were studied pathologically.

When the infarct was two days old, neither dose of cortisone had disturbed healing appreciably. By four and six days cortisone, in the dose of 2.5 mg. per kilogram of body weight, had inhibited slightly the removal of necrotic muscle fibers as compared with untreated controls. This delay could not be appreciated grossly. At all other intervals studied, infarcts in the animals receiving this dose of cortisone did not differ from the control group.

Cortisone, in a dose of 10 mg. per kilogram of body weight daily, delayed to a small but noticeable degree the healing of infarcts at 4, 6, 12 and 21 days. At four days the delay in resorption of necrotic muscle was no greater than in the group receiving the smaller dose of cortisone and could not be appreciated grossly. At six days the delay in healing was somewhat greater than in the group receiving the smaller dose of cortisone and could be appreciated grossly. The inhibition noticed at four and at six days was the greatest in degree among all the experiments. At 12 days a difference, minor but yet appreciable, was noted between the infarcts in the control group and in the group receiving the larger dose of cortisone. On microscopic examination removal of necrotic muscle and scar formation had been delayed in the latter group as compared to controls. At 21 days the larger dose of cortisone questionably delayed healing, whereas at 60 days all infarcts in the control and cortisone-treated series appeared to be equally well healed.

SUMMARIO E CONCLUSIONES IN INTERLINGUA

In 36 canes bastardes infarctos myocardiac esseva produciti per ligari le descendente arteria coronari anterior. Le canes esseva dividiti in tres gruppos equal. Duo gruppos recipieva diurne injectiones intramuscular de 2,5 et 10 mg respectivemente de cortisona per kg de pesocorpore. Le tertie grupo recipieva injectiones de un solution de controlo. A periodos de 2, 4, 6, 12, 21, e 60 dies post le production del infarctos, sex canes (duo de cata grupo) esseva sacrificate e studiate pathologicamente.

Quando le infarctos habeva un etate de duo dies, ni le un ni le altere dosage de cortisona habeva disturbate le processo curative a un grado appreciabile. Post quatro e sex dies, le dosage de 2,5 mg cortisona per kg de pesocorporee habeva exercite un leve effecto inhibitive super le resorption del necrotic fibras muscular in comparation con le stato del animales de controlo. Iste retardation non esseva grossiermente appreciabile. A omne le altere periodos studiate, le infarctos del animales de iste grupo (2,5 mg cortisona per kg pesocorpore) non se distingueva de illos del grupo de controlo.

In le dosage de 10 mg per die per kg pesocorporee, cortisona retardava a un leve sed sensibile grado le curation del infarctos post 4, 6, 12, e 21 dies. Post quatro dies le effecto inhibitive del cortisona super le resorption de musculo necrotic non esseva plus grande in iste grupo que in le grupo con le plus parve dosage. Illo non esseva grossiermente appreciabile. Post sex dies le retardation de' curation
EFFECT OF CORTISONE ON MYOCARDIAL INFARCTS

esseva alique plus grande in iste gruppo que in le gruppo con le plus parve dosage e poteva esser grossieramente appreciate. Le inhibition notate post quatro e sex dies eseva le plus grande inter omne le experimentos. Post 12 dies un minor sed ancora appreciabile differ-rentia eseva notate inter le infarctos del gruppo de controlo e illos del gruppo con le plus grande dosages de cortisona. Le examine microscopic revelava in iste ultime gruppo un retardation del resorption de musculo necrotic e del cicatrisation. Post 21 dies le retardate curation causate per le plus grande dose de cortisona eseva questionabile. Post 60 dies omne le infarctos in tanto le gruppo de controlo como etiam le gruppis tractate con cortisona pareva equalmente ben curate.

REFERENCES


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