EFFECT OF OPIOID PEPTIDE ON HEALING OF AN EXPERIMENTAL MYOCARDIAL INFARCT

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Opioid peptides, a new group of biologically active substances, have been shown to stimulate repair processes in the gastrointestinal tract [5], nerve tissue [1], and skin [2]. On this basis the universality of this action of these compounds, due to activation of the protective forces of the organism, has been postulated [3]. In the investigation described below the effect of one of the opioid peptides, a synthetic Leu-enkephalin analog known as dalargin (Dala-Gly-Phe-Leu-Arg) on the healing of an experimental myocardial infarct (MI) was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 62 male chinchilla rabbits weighing 2-2.5 kg. Myocardial necrosis was produced by ligating the descending branch of the left coronary artery. The animals were killed at the peak time of healing of necrosis (3rd day after the operation) and on its completion (7th day). At each of these times of the experiment three groups of animals were used: 1) control (ligation of the coronary artery without treatment), 2) preliminary injection of dalargin for 2 days before the operation and thereafter until sacrifice in a daily dose of 10 μg/kg, 3) animals starting to receive dalargin 30 min after the operation. Transverse blocks of myocardium passing through the center of necrosis were fixed in alcohol-formol and embedded in paraffin wax. Sections were stained: with hematoxylin and eosin to assess the state of the cells, by Masson's method to detect collagen and muscle fibers, with alcian blue to evaluate the ground substance by demonstrating acid glucosaminoglycans. The above-mentioned components in the zone of necrosis were determined quantitatively by the dot method and their content in a standard area of section was estimated in per cent. Material for electron microscopy was treated in the usual way. The dimensions of MI were determined on the 7th day of the experiments by the method in [6] (the results are given in per cent of the total weight of the heart). The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Light Microscopy. Data on changes in the content of ground substance of the connective tissue, collagen, and unabsorbed muscle fibers in the zone of necrosis and also the number of cells in the center and at the periphery of necrosis on the 3rd day of the experiments are given in Table 1. All these parameters showed significant changes under the influence of dalargin. If the parameters determined in the group of untreated animals were taken as 100%, the quantity of ground substance was reduced by 32.5% in the rabbits of group 2 and by 28.3% in the rabbits of group 3 compared with the control ($P < 0.01$). The collagen content was increased by 27.9% ($P < 0.001$) and 25.1% ($P < 0.05$), respectively. The number of cells both at the periphery and in the center of the zone of necrosis also was increased statistically significantly. The area occupied by unabsorbed muscle fibers showed a tendency to decrease in the treated animals of both groups, but the results for this parameter were not significant.

Size of MI. In rabbits of the experimental groups MI had a definite tendency to decrease in size, but the difference from the control was not statistically significant (control 9.8 ± 1.0%, group 2, 8.5 ± 1.4%, group 3, 7.5 ± 1.7%, $P < 0.1$).

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TABLE 1. Content of Principal Components of Zone of Myocardial Necrosis during Dalargin Therapy (in % of standard area)

Component	Control	Administration of dalargin	
		preliminary	after 30 min
Ground substance	28,6±1,7	19,3±1,5	20,5±1,5
P		<0,01	<0,01
Collagen	18,3±0,3	23,4±0,4	22,9±2,0
P		<0,001	<0,05
Muscle fibers	22,8±5,5	17,2±2,0	16,7—4,4
P		>0,1	>0,1
Cellular elements:			
In center of necrosis	32,2±1,2	41,0±1,5	40,2±1,0
P		<0,01	<0,01
At periphery of necrosis	41,0±1,9	48,2±1,5	51,6±0,8
P		<0,02	<0,01

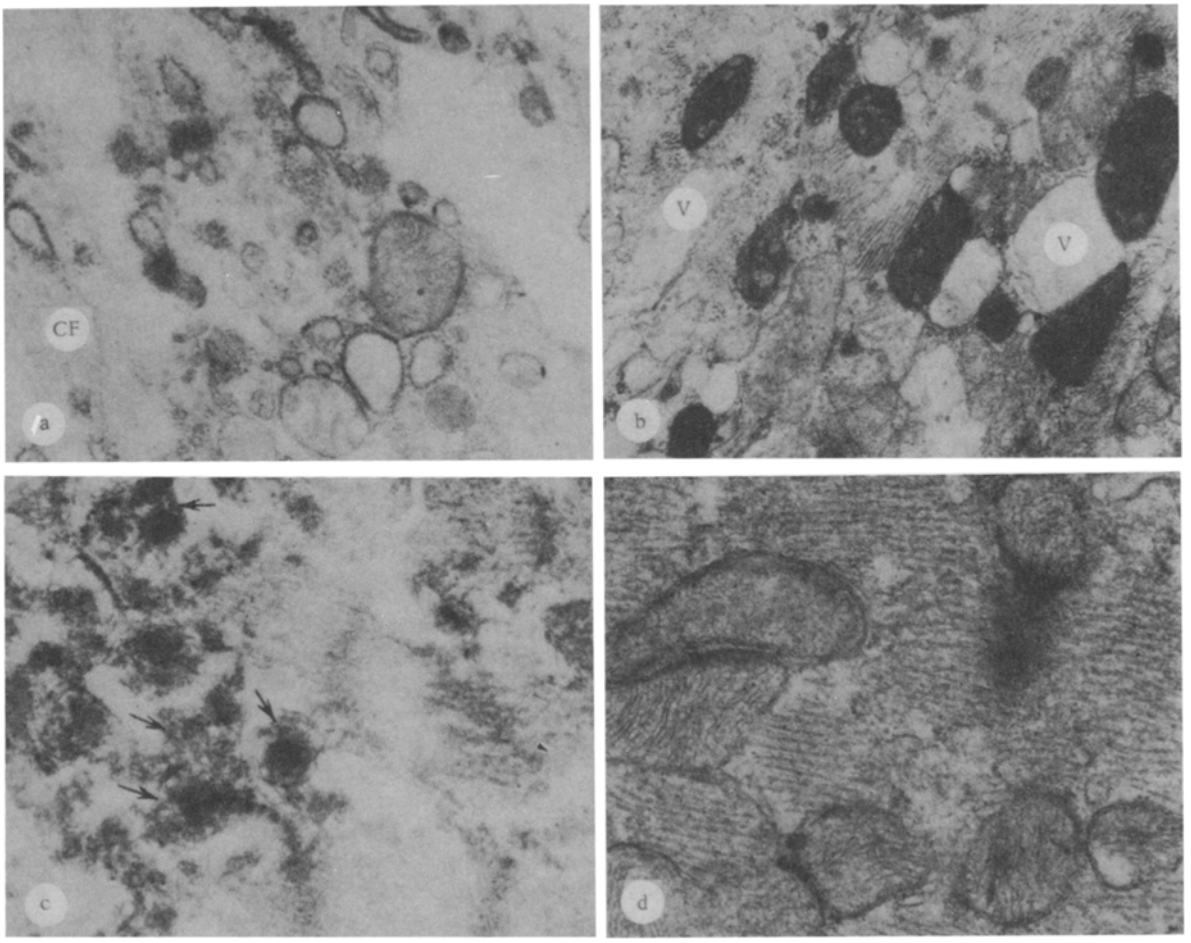

Fig. 1. Ultrastructure of rabbit myocardium in zone of MI on 7th day after operation: a-c) untreated animals, d) series of experiments with injections of dalargin. a) Cell debris in connective-tissue space of myocardium. CF) Collagen fibrils. 30,000; b) Vacuoles (V) in a cardiomyocyte. 30,000; c) Electron-dense clumps of material (arrows) in cytoplasm of a cardiomyocyte. 35,000×; d) Well preserved ultrastructures of a cardiomyocyte. 50,000×.

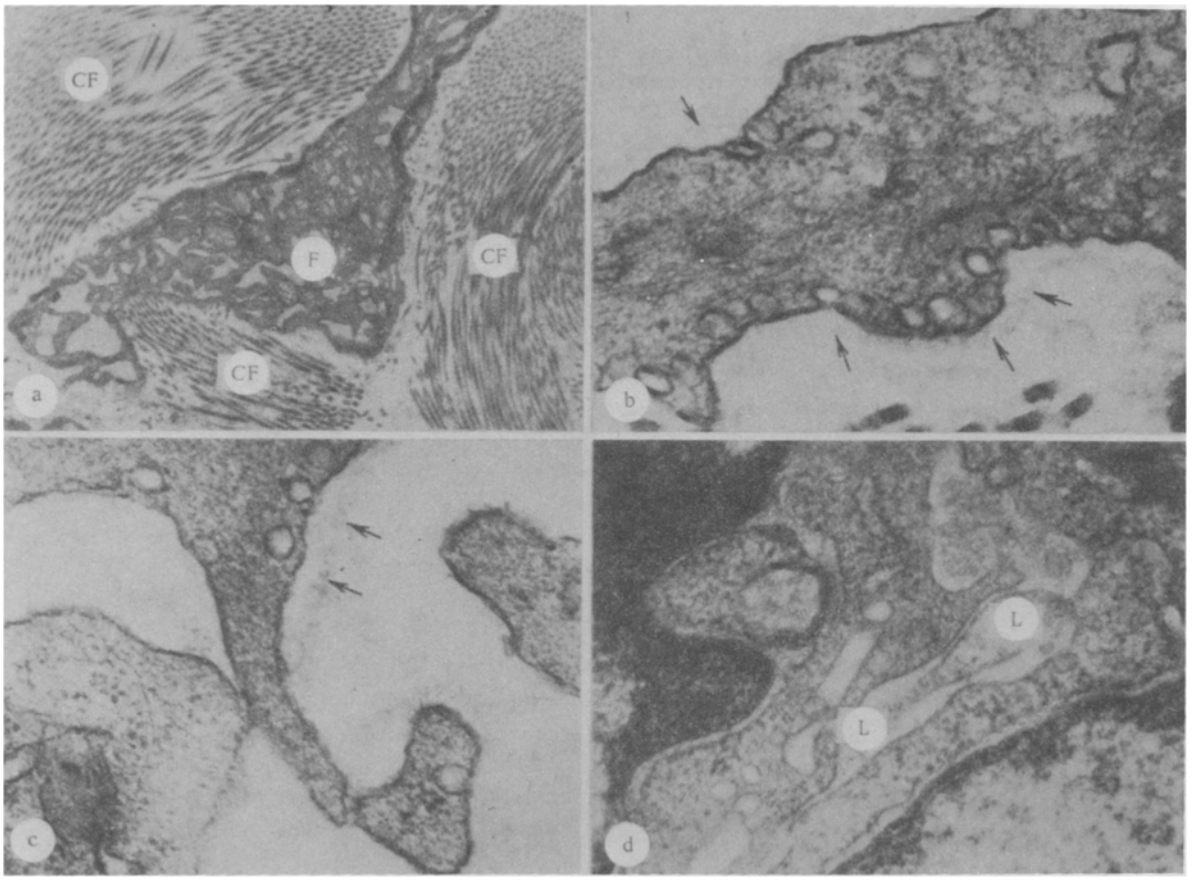

Fig. 2. Ultrastructure of myocardial connective-tissue cells in rabbits treated with dalargin in zone of MI on 7th day of experiment. a) Numerous collagen fibrils (CF), a fibroblast (F) in an active state. 7000×; b) Long cell process containing many micropinocytotic vesicles and microfilaments and surrounded by a basement membrane (arrows). 65,000×; c) Junction between processes of two cells, ultrastructure of one of which is similar to that shown in Fig. 1b. Basement membrane in certain places (arrows). 65,000×; d) Cells in connective-tissue space form vascular structure with narrow, irregularly shaped lumen (L). 60,000×.

Electron Microscopy. Considerable destruction of cells, both muscular and connective-tissue, was observed in the zone of infarction in the control. As a result of massive cell death, groups of intracellular organelles, mingled with collagen fibrils, were often found in the connective-tissue spaces (Fig. 1a). Destructive changes of varied degree of severity were found in cardiomyocytes in the zone of infarction. Translucency of the matrix and reduction of the cristae were found in the mitochondria. Vacuoles appeared in the cytoplasm (Fig. 1b). In some cases the mitochondria had completely lost their outer and inner membranes and were converted into clumps of electron-dense material (Fig. 1c). The contractile system of the cardiomyocytes showed disorientation and lysis of the plasmalemma in platelets. Processes of destruction were much less marked in the experimental material. No concentrations of organelles were present in the connective-tissue spaces. The degree of preservation of the cardiomyocytes was better in the series of experiments with preliminary administration of dalargin (Fig. 1d). Damage to the mitochondria and contractile system was minimal, and cisterns of the rough endoplasmic reticulum appeared in the cytoplasm of the cardiomyocytes, evidence of increased synthetic activity of the cells. Large bundles of collagen fibrils were found (Fig. 2a). All these phenomena ensure more effective scar formation.

Dalargin also has another interesting property. Long and narrow processes of cells were seen in the postinfarct scars of the treated animals (Fig. 2b). Since they have a well developed basement membrane and many micropinocytotic vesicles and microfilaments, these cells may be identified as endothelial cells or pericytes.

The angiogenic nature of these cells also was confirmed by their tendency to form intercellular junctions and vascular structures with a slit-like lumen (Fig. 2c, d). Some of these cells were possibly myofibroblasts. Their presence in the zone of necrosis has been demonstrated in rats with experimental MI [4]. Myofibroblasts synthesize type III collagen and elastin, which increases the strength and elasticity of scar tissue. The results suggest that dalargin is a stimulator of capillary growth and of myofibroblast formation.

Dalargin thus accelerates the healing of experimental MI, in agreement with the original hypothesis [3].

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