Cannabidiol, a nonpsychoactive *Cannabis* constituent, protects against myocardial ischemic reperfusion injury

Ronen Durst,[1] Haim Danenberg,[1] Ruth Gallily,[2] Raphael Mechoulam,[3] Keren Meir,[4] Etty Grad,[1] Ronen Beeri,[1] Thea Pugatsch,[1] Elizabet Tarsish,[1] and Chaim Lotan[1]

[1]*Cardiology Department and* [4]*Pathology Department, Hadassah Hebrew University Medical Center,* [2]*Lautenberg Center for General and Tumor Immunology, and* [3]*Department of Medicinal Chemistry and Natural Products, Pharmacy School, Hebrew University Medical School, Jerusalem, Israel*

Durst R, Danenberg H, Gallily R, Mechoulam R, Meir K, Grad E, Beeri R, Pugatsch T, Tarsish E, Lotan C. Cannabidiol, a nonpsychoactive *Cannabis* constituent, protects against myocardial ischemic reperfusion injury. *Am J Physiol Heart Circ Physiol* 293: H3602–H3607, 2007. First published September 21, 2007; doi:10.1152/ajpheart.00098.2007.—Cannabidiol (CBD) is a major, nonpsychoactive *Cannabis* constituent with anti-inflammatory activity mediated by enhancing adenosine signaling. Inasmuch as adenosine receptors are promising pharmaceutical targets for ischemic heart diseases, we tested the effect of CBD on ischemic rat hearts. For the in vivo studies, the left anterior descending coronary artery was transiently ligated for 30 min, and the rats were treated for 7 days with CBD (5 mg/kg ip) or vehicle. Cardiac function was studied by echocardiography. Infarcts were examined morphometrically and histologically. For ex vivo evaluation, CBD was administered 24 and 1 h before the animals were killed, and hearts were harvested for physiological measurements. In vivo studies showed preservation of shortening fraction in CBD-treated animals: from 48 ± 8 to $39 \pm 8\%$ and from 44 ± 5 to $32 \pm 9\%$ in CBD-treated and control rats, respectively ($n = 14$, $P < 0.05$). Infarct size was reduced by 66% in CBD-treated animals, despite nearly identical areas at risk (9.6 ± 3.9 and $28.2 \pm 7.0\%$ in CBD and controls, respectively, $P < 0.001$) and granulation tissue proportion as assessed qualitatively. Infarcts in CBD-treated animals were associated with reduced myocardial inflammation and reduced IL-6 levels (254 ± 22 and $2,812 \pm 500$ pg/ml in CBD and control rats, respectively, $P < 0.01$). In isolated hearts, no significant difference in infarct size, left ventricular developed pressures during ischemia and reperfusion, or coronary flow could be detected between CBD-treated and control hearts. Our study shows that CBD induces a substantial in vivo cardioprotective effect from ischemia that is not observed ex vivo. Inasmuch as CBD has previously been administered to humans without causing side effects, it may represent a promising novel treatment for myocardial ischemia.

ischemia-reperfusion; myocardial infarction; cannabinoids; pharmacotherapy

CANNABINOIDS ARE NATURAL and synthetic compounds structurally or pharmacologically related to the constituents of the plant *Cannabis sativa* or to the endogenous agonists (endocannabinoids) of the cannabinoid CB_1 and CB_2 receptors (for recent reviews see Refs. 17, 22, and 24). In a rat model, it was shown that anandamide, an endogenous cannabinoid (8), has a physiological triphasic effect on the cardiovascular system consisting of an initial reduction in heart rate (HR) and blood pressure followed by a brief pressor response and then a prominent reduction of blood pressure and HR (29). It has also been shown that endocannabinoids have a protective effect against myocardial ischemia and can help preserve coronary endothelial function during ischemia (15, 27, 28). These effects are receptor mediated and can be inhibited by specific CB_1 and CB_2 receptor blockers (15, 27, 28). In one study, a potent CB_1 and CB_2 receptor agonist, HU-210, was shown to substantially reduce the myocardial necrotic zone after left anterior descending coronary artery (LAD) ligation (28); in another trial, HU-210 was shown to significantly reduce the ischemic arrhythmic effect in treated animals. These effects were mediated by the CB_2 receptor (15, 27). Other studies have shown that the cardioprotective effect of heat and ischemic preconditioning is mediated, partly at least, by CB_2 receptors and can be abolished by CB_2 receptor antagonists (3, 13). HU-210 was also shown to increase coronary blood flow and to be associated with reduced remodeling in infarcted rat hearts (31, 32). These results indicate a possible role for the endocannabinoid system in stress-induced preconditioning.

Cannabidiol (CBD) is a major cannabinoid constituent of *Cannabis* (18). In contrast to tetrahydrocannabinol, CBD binds very weakly to CB_1 and CB_2 receptors (18, 24). Contrary to most cannabinoids, CBD does not induce psychoactive or cognitive effects. CBD has been shown to have anti-inflammatory properties (6, 16, 33). CBD (together with tetrahydrocannabinol) has been successfully tested in a few preliminary human trials related to autoimmune diseases such as rheumatoid arthritis (2) and multiple sclerosis (4, 26, 38) and is well tolerated by humans (7, 18, 24).

The mechanisms of action of CBD remain obscure. It has a mild effect on vanilloid VR_1 receptors (1, 25). Recently, it was proposed that the anti-inflammatory effects of CBD might be mediated by enhancement of adenosine signaling through inhibition of its uptake (5). This effect apparently involves the adenosine A_{2A} receptor (5).

In view of the known cardioprotective effect of cannabinoids, the reduction in infarct size induced by adenosine receptor activation (34), the enhanced A_{2A} receptor signaling by CBD (5), the anti-inflammatory effect of CBD, which might prevent immune-related myocardial injury (14), and its lack of psychotropic or cognitive effects (24), we studied the possible cardioprotective effect of CBD on ischemic rat hearts. We also tested whether any protective effect is exerted directly on the myocardium or via mediators of immune mechanisms.

Address for reprint requests and other correspondence: R. Durst, Cardiac Echo Lab, Massachusetts General Hospital, Blake 2, Rm. 255, 55 Fruit St., Boston, MA 02114 (e-mail: rdurst@partners.org).

Table 1. *Baseline and 7-day parameters of CBD and control animals*

| | CBD (*n* = 14) | | Control (*n* = 14) | | *P* Value | | | |
	Day 0 (a)	*Day 7 (b)*	*Day 0 (c)*	*Day 7 (d)*	*a* vs. *b**	*c* vs. *d**	*a* vs. *c*	*b* vs. *d*†
Body wt, g	326.79±3.36	314.57±8.67	326.07±4.32	319.71±8.14	<0.01	0.015	0.63	0.11
HR, beats/min	308±27	304±22	314±25	310±29	0.5	0.45	0.54	0.39
LVEDD, cm	0.70±0.06	0.72±0.05	0.67±0.10	0.72±0.05	0.14	0.07	0.32	0.91
LVESD, cm	0.36±0.08	0.43±0.07	0.37±0.07	0.49±0.06	<0.01	<0.01	0.76	0.041
SF, %	48±8	39±8	43±5	32±9	0.02	<0.01	0.26	0.03

Values are means ± SE. CBD, cannabidiol; HR, heart rate; LVEDD, left ventricular (LV) end-diastolic diameter; LVESD, LV end-systolic diameter; SF, shortening fraction. *Paired analysis. †For *t*-test, percent change from baseline was used as the variable.

METHODS

Animals. Male Sprague-Dawley rats (275–300 g body wt) were used: 7 animals were used in the CBD-treated and vehicle-injected arms of the Langendorff isolated rat heart model, and 14 animals were used in the CBD-treated and vehicle-injected arms of the whole animal study. The Institutional Animal Study Review Board approved the study.

CBD. CBD was extracted from *Cannabis* resin (hashish) and purified as previously described (9). The crystalline CBD (66–67°C melting point) presented a single peak on gas chromatography analysis and is ≥99% pure (9). CBD was dissolved in absolute ethanol, with an equal volume of a detergent (Cremophor), and the solution was mixed until homogeneous. Saline was added to a final ratio of 1:1:18 ethanol-detergent-saline. The final concentration of 5 mg·kg body wt^{-1}·day^{-1} was injected intraperitoneally.

In vivo LAD ligation procedure. Rats were anesthetized with 10% ketamine-2% xylazine (0.85:0.15, 0.1 ml/kg im), intubated, and ventilated with a small animal respirator (Harvard). The heart was exposed via left sternotomy, and the LAD was reversibly occluded for 30 min with a snare fashioned from an encircling silk ligature and a section of 5-Fr feeding tube. Coronary occlusion and reperfusion were verified by visual inspection. After reperfusion, the released ligature was left in situ. The chest was closed in layers with uninterrupted sutures. Rats were extubated, lost body fluids were replaced by subcutaneous administration of 30–50 ml of saline, and the animals were treated with rymadil (5 mg/kg ip) analgesia for 3 days. The animals were randomized to receive intraperitoneal injections of 5 mg/kg CBD or solvent vehicle as control 1 h before the procedure and every 24 h thereafter for 7 days until they were killed. The drug and vehicle solutions were prepared in separate laboratories to ensure that the staff (e.g., echocardiography and pathology) was blinded to the treatment arm. After 7 days, animals were reanesthetized and ventilated as described above. The LAD was permanently reoccluded, and the heart was harvested for further analysis.

Ex vivo isolated Langendorff rat heart LAD ligation. To assess the direct CBD effect on the heart, we used the isolated Langendorff rat heart model (19). Animals were intraperitoneally injected with 5 mg/kg CBD or solvent 24 and 1 h before heart removal. At 30 min before heart removal, unfractionated heparin (750 IU, 0.15 ml ip) was injected into the rat. Animals were anesthetized with 0.6–0.8 ml of 20% pentobarbital sodium by intraperitoneal injection. After it was removed, the heart was placed in cold (4°C) saline-heparin solution (100 ml of saline and 750 IU of heparin). The aorta was cannulated, and the heart was perfused with modified Krebs-Henseleit solution under a constant pressure of 90 mmHg. A water-filled latex balloon was inserted into the left ventricular (LV) cavity via the left atrium, coupled to a pressure transducer, to record LV developed pressures (LVDP). A 50-μl Hamilton syringe was used to adjust LV end-diastolic pressure (LVEDP) to 5–10 mmHg. Coronary flow (CF) was measured throughout the procedure by collection of the effluent. HR was set to 300 beats/min by an external pacemaker attached to the left auricle to adjust for possible differences in HR between the treatment

groups. LVDP was continuously recorded on a polygraph. After 30 min of stabilization, a 4-0 silk suture was placed under the LAD below an imaginary interauricular line and then threaded through a button-like snare. This allows ligation for a prolonged period without harm to the heart. The LAD was occluded for 45 min; then the snare was released, and reperfusion through the LAD was allowed for 45 min. After 45 min of reperfusion, the LAD was reoccluded and the percent infarct size of the area at risk was determined (see below).

Echocardiography. Echocardiographic imaging was performed using a GE Vivid3 platform equipped with a 13-MHz linear epiaortic transducer (General Electric, Haifa, Israel). The probe was positioned in a left parasternal position, and two-dimensional imaging of the heart in the short axis was performed using a high frame rate. This
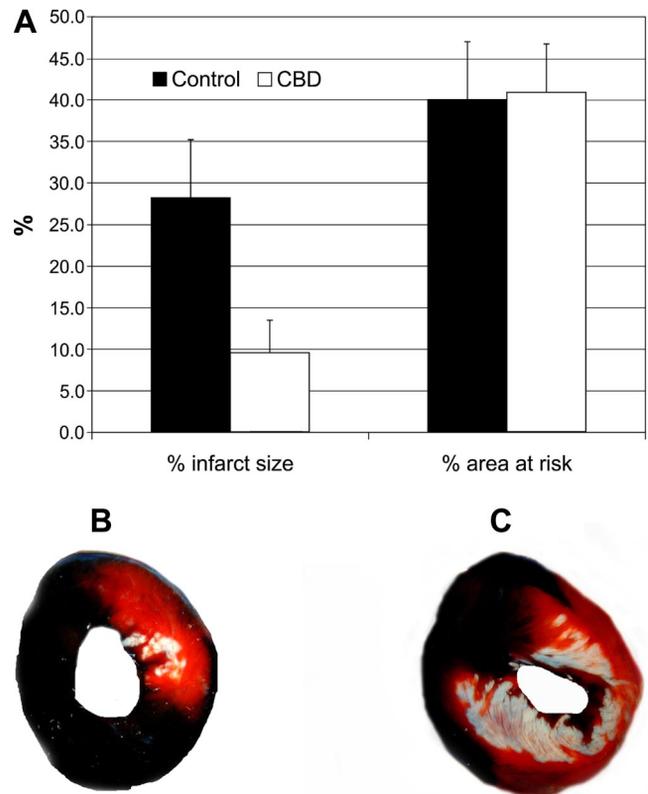

Fig. 1. *A*: percent area at risk of the left ventricle (LV) and percent infarct size of the area at risk in cannabidiol (CBD)-treated and control animals. Infarct size was measured as percentage of area at risk by planimetry using triphe-nyltetrazolium chloride (TTC) staining. Animals were treated with CBD (5 mg/kg) 1 h before and 7 days after ligation of the left anterior descending coronary artery or were untreated. *P* < 0.01, CBD vs. control (by unpaired 2-tailed *t*-test). *B* and *C*: TTC staining in a CBD and a control animal, respectively. Area at risk is stained red and infarct is white.
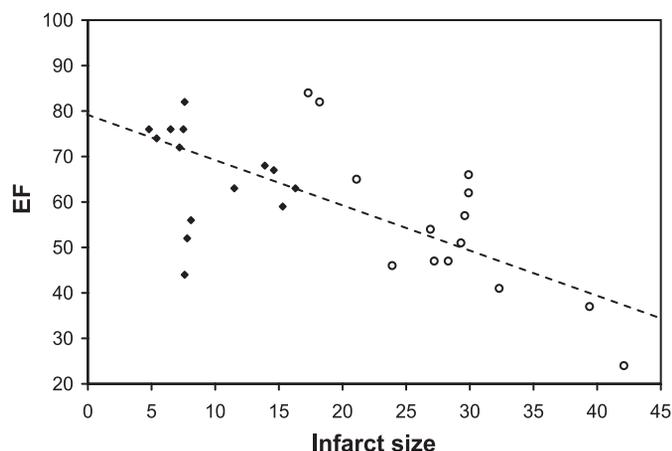
Fig. 2. Correlation between infarct size and ejection fraction (EF). ◆, CBD; ○, control. $R^2 = 0.45$ ($P < 0.05$), $\beta = -0.9$. Dashed line represents estimated linear correlation.

image was used to guide an M-mode cursor down the medial axis of the LV. Measurements were performed in triplicate using the leading edge convention for myocardial borders. The images were acquired with HR at 250–350 beats/min.

The following parameters were measured: LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD). Shortening fraction (SF) was calculated using these variables.

Infarct size measurements and histopathology. Measurements were performed as previously described (20, 21, 37). Briefly, 0.5–1 ml of 1% Evans blue solution in saline was slowly infused retrogradely through the aorta into the coronary arteries to mark the area at risk (not stained). The heart was then frozen (−20°C) for 1 h in aluminum foil and cut into 2-mm-thick transverse sections from the occlusion area (LAD) to the apex (usually 4–6 slices). The slices were stained by incubation at 37°C for 15 min in 1% (wt/vol) triphenyltetrazolium chloride (TTC) in phosphate buffer (pH 7.4) and fixed in 10% (vol/vol) formaldehyde solution. The stained slices were photographed, and the area of LV at risk and the area of infarcted tissue in the risk zone were determined by planimetry using Adobe Photoshop software. Subsequently, tissue slices were fixed in 5% ammonium hydrochloride, embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin and eosin and Masson's trichrome. The extent of the inflammatory response was graded on a scale of 1–3 by an experienced pathologist who was blinded to the treatment groups.

Serum TNF-α, IL-6, and C-reactive protein measurements. Rats were bled 7 days after coronary ligation. Sera were separated and frozen. Concentration of TNF-α, Il-6, and C-reactive protein (CRP) in the sera was measured by commercial ELISA kit according to the manufacturer's protocols.

ELISA reagents for TNF-α were purchased from Biosource (Camarillo, CA), for IL-6 from AbD Serotec (Kidlington, UK) and Prospect (Rehovot, Israel), and for CRP from Immunology Consultants Laboratory (Newberg, OR).

Statistical analysis. Unpaired Student's *t*-test was used to compare baseline data between groups. Within-group outcomes were compared with baseline using paired *t*-test analysis. Between-group outcome
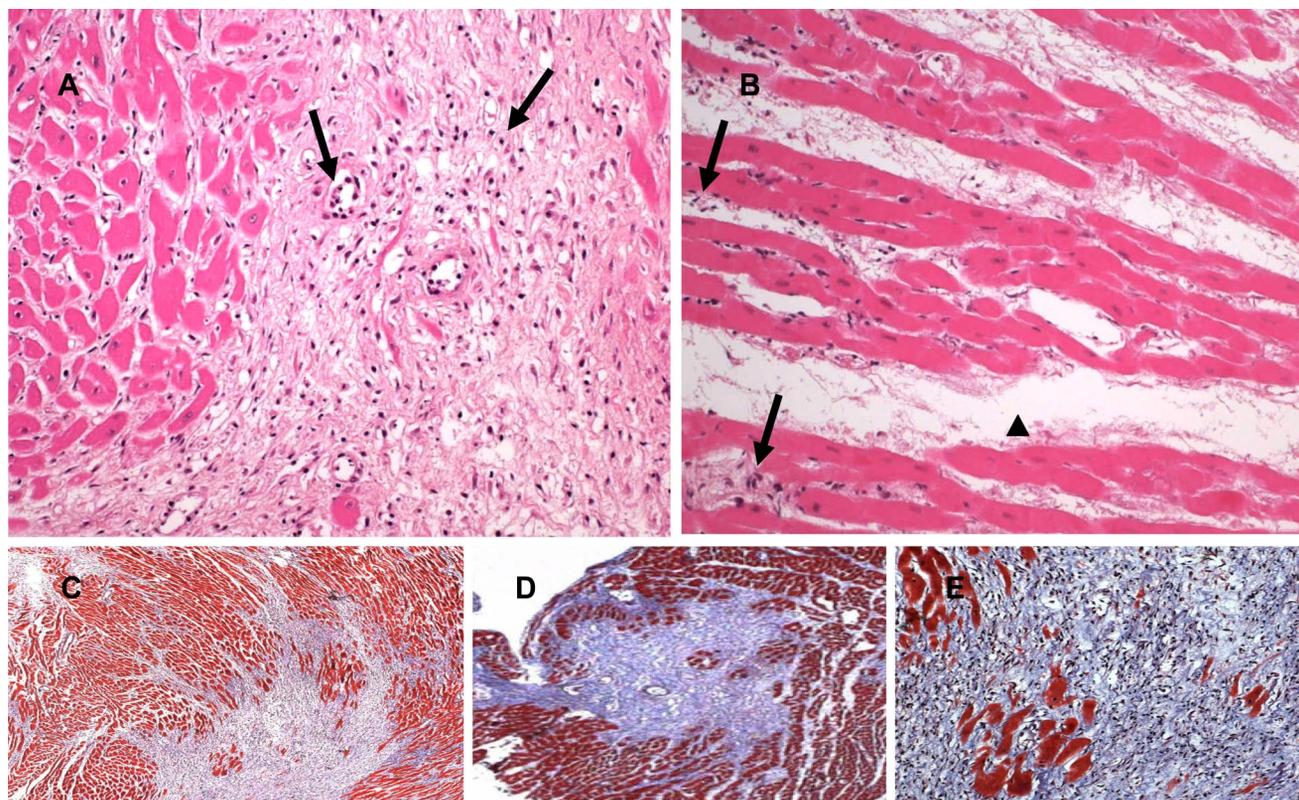

Fig. 3. Histological analysis of CBD-treated and control rats. *A*: marked mononuclear infiltration (arrows) and early collagen fiber deposition in a typical control animal. Magnification ×40, hematoxylin and eosin. *B*: almost total lack of inflammation in an animal treated with CBD. Note occasional mononuclear cells (arrows) in viable myocardial interstitium and marked edema (arrowhead). Magnification ×40, hematoxylin and eosin. *C*: early collagen deposition in infarcted area in a control animal. Magnification ×40, Masson's trichrome. *D*: early collagen deposition in infarcted area in a CBD-treated animal. Staining was similar in CBD and control groups. Magnification ×40, Masson's trichrome. *E*: replacement of necrotic tissue by early fibrovascular granulation tissue in a control heart; findings were identical in CBD-treated hearts (data not shown). Magnification ×20, Masson's trichrome.

analysis was performed using percent change from baseline and unpaired *t*-test. Linear correlation was used to compare infarct size and ejection fraction (EF).

RESULTS

Fourteen CBD- or vehicle-treated animals completed the experimental design. Four control animals and three CBD-treated animals died before *day 7*. Weight, LVEDD, LVESD, and SF of the animals that survived to *day 7* are presented in Table 1.

All animals showed significant weight loss 7 days after the procedure: from 326.8 ± 3.4 g at baseline to 314.6 ± 8.7 g (CBD) and from 326.1 ± 4.3 g at baseline to 319.7 ± 8.1 g (controls; $P < 0.05$). As expected, a significant reduction in SF was observed in all animals after LAD ligation: from $48 \pm 8\%$ at baseline to $39 \pm 8\%$ (CBD) and from $44 \pm 6\%$ at baseline to $32 \pm 9\%$ (controls; $P < 0.05$). The LV area at risk and infarct size as a percentage of area at risk are presented in Fig. 1. The area at risk was similar in CBD and control animals. However, the mean infarct size was significantly and remarkably reduced in CBD-treated animals ($9.6 \pm 3.9\%$ vs. $28.2 \pm 7.0\%$ in CBD and control arms, respectively, $P < 0.001$). Thus a relative reduction of 66% in the infarcted zone was observed in the CBD-treated animals. The ejection fraction on *day 7* negatively correlated with infarct size ($R^2 = 0.45$, $\beta = -0.9$; Fig. 2). To further determine whether the reduction in infarct size is related to an improved remodeling process, LV function of animals with the largest infarct in the CBD group was compared with that of animals at the lower end of infarct size range in the control groups. In these animals, the area of the infarct ranged from 16 to 20% in both groups. There was no difference in LVEDD on *day 7* (7.4 ± 0.8 vs. 7 ± 0.6 mm respectively) or in SF (33% vs. 37% respectively) between CBD-treated animals and controls. Although this comparison is not statistically powerful, it might suggest that the CBD effect is mainly due to the reduction in infarct size, rather than major changes in myocardial contractility or remodeling processes.

Hearts from each group were stained with hematoxylin and eosin and Masson's trichrome (Fig. 3). The inflammatory infiltrate was graded on a scale of 1–3 by an expert pathologist on the basis of the number of leukocytes infiltrating the border of the infarcted zone. The inflammatory response in the control animals was graded 2–3 compared with 1+ or 0 in the CBD-treated animals (Fig. 3, *A* and *B*), indicating elimination of the inflammatory process in the treated animals. However,

Table 2. *Infarct size and LVDP as percentage of baseline during ischemia and after reperfusion in CBD and control animals in an isolated heart preparation*

	CBD ($n = 7$)	Vehicle ($n = 7$)
Infarct size, %	11.9 ± 9.5	13.2 ± 8.1
LVDP, %		
Ischemia	49 ± 13	48 ± 12
Reperfusion	61 ± 3	62 ± 4
Coronary flow during ischemia, %	56 ± 18	52 ± 9

Values are means $\pm$ SE. Infarct size was measured as percentage of area at risk and LV developed pressure (LVDP) as percentage of baseline. None of the results reached statistical significance.

Table 3. *TNF-α, IL-6, and CRP in sera of CBD and control aniimals*

	TNF-α, pg/ml	IL-6, pg/ml	CRP, ng/ml
CBD	10 ± 1.3	254 ± 22	46 ± 4.5
Control	13 ± 1.6	2812 ± 500	53 ± 3.4
P value	NS	< 0.05	NS

Values are means $\pm$ SE of 8 rats. CRP, C-reactive protein. IL-6 was significantly reduced in CBD animals, indicating a significant anti-inflammatory effect of CBD. NS, not significant.

granulation tissue formation with early collagen deposition was similar in the treated and control animals (Fig. 3, *D* and *E*).

To understand whether the CBD cardioprotective effect is due to a direct effect on the myocardium or, rather, is mediated via secondary mechanisms, in vitro isolated heart studies were performed. Infarct size, area at risk, LVDP, and coronary flow (CF) after coronary ligation, presented as percentage of the baseline values after stabilization, are shown in Table 2. No differences in any of the parameters were observed between CBD-treated and control animals.

In view of the lack of CBD effect on isolated heart, IL-6, CRP, and TNF-α were measured on *day 7* (Table 3). IL-6 was significantly lower in the sera of CBD-treated than control
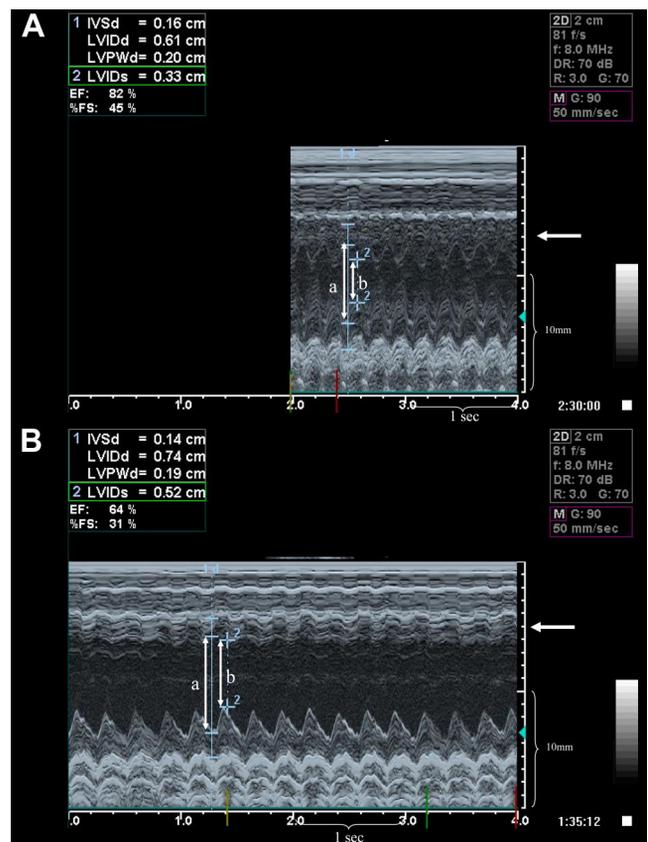

Fig. 4. *A*: CBD-treated animal with preserved LV function and preserved motion of the anterior wall (horizontal arrow) and a corresponding shortening fraction (SF) of 45%. *B*: M-mode echocardiography of a sham-treated rat with reduced SF and reduced motion of the anterior wall (horizontal arrow) and a corresponding SF of 31%. Double-headed arrows: LV end-diastolic diameter (*a*) and LV end-systolic diameter (*b*). IVS$_d$, interventricular septum thickness at diastole; LVID$_s$ and LVID$_d$, LV internal diameter at systole and diastole, respectively; LVPW$_d$, LV posterior wall thickness at diastole.

animals (254 ± 22 vs. $2{,}812 \pm 500$ pg/ml, $P < 0.001$). CRP and TNF-α did not differ significantly between the two groups on *day 7*.

DISCUSSION

Our study demonstrates that CBD has a significant in vivo cardioprotective effect against myocardial ischemia induced by LAD ligation, with reduction of infarct size measured as percentage of the area at risk by TTC staining. This was accompanied by a significant reduction in the decrease in LV function, which is observed after coronary occlusion, measured by echocardiography (Fig. 4). The EF after 7 days correlated pretty well with infarct size, indicating that the preservation of EF in treated animals is mainly due to reduced infarct size. This fact is further supported by comparing animals with similar infarct sizes from both groups.

The reduction of the infarct size was associated with a qualitative reduction in inflammatory infiltration of the ischemic and necrotic myocardial zones. CBD did not affect early collagen formation as detected by Masson's trichrome staining. These findings were associated with a significant reduction in serum IL-6 levels. We did not detect a significant effect of CBD on infarct size, cardiac contractility, and coronary blood flow in the in vitro isolated rat heart model. Because CBD has known anti-inflammatory effects (5, 6), it is plausible that the in vivo cardioprotective effect of CBD may be mediated by a systemic immunomodulatory effect, such as the reduction in IL-6 levels. A similar concept was recently proposed as the mechanism of action of a newly synthesized molecule, 1,6-bis(phosphocholine)-hexane, which binds to CRP and prevents its interaction with various inflammatory ligands. Complement activation was also noted (23). Infarct size is reduced from 24% in CRP-treated rats 5 days after LAD ligation to 17% by 1,6-bis(phosphocholine)-hexane (23). CRP is assumed to play a role in the innate immunity and immune activation in damaged myocardium (14). Thus anti-inflammatory agents such as CBD and 1,6-bis(phosphocholine)-hexane might have a role in preventing immune-related myocardial injury. It should be stressed that the in vivo and in vitro models were not fully comparable. The CBD treatment duration and the reperfusion period were shorter in the isolated heart model. Also, the ischemic period was longer. These experimental differences may explain the difference in infarct size between the in vivo and ex vivo experiments. However, the CBD effect in vivo was very robust, whereas it was essentially nonexistent in the ex vivo experiment (Table 2), indicating that CBD did not have an equivalent effect in vivo and ex vivo.

Previous studies have shown that cannabinoids, which act on cannabinoid receptors, reduce infarct size and protect the heart from ischemia-related arrhythmia (15, 27, 28). Also endogenous cannabinoids were found to be involved in preconditioning induced by heat and ischemia. Furthermore, such cannabinoids have vasodilatory effects and can improve endothelial function (3, 13). In all these studies, however, the effects of these cannabinoids, either endogenous or synthetic, were shown to be mediated by activation of the CB_1 or CB_2 receptor. Inasmuch as CBD does not act via the known cannabinoid receptors and does not have direct hemodynamic effects, its mechanism of action presumably differs from that of the cannabinoid receptor agonists. Evidence of transactivation of

adenosine receptors by CBD was recently published (5). This effect was associated with the anti-inflammatory properties of CBD.

Adenosine receptors seem to play a role in protecting the heart (34). Adenosine A_1 and A_3 receptors have been most intensively studied, and the A_{2A} receptor may also play a role. The A_3 receptor seems to have multiple beneficial effects on ischemia-reperfusion injury, including modulation of necrotic and apoptotic cell death and enhancement of contractile function (11). A_1 receptor overexpression increases myocardial resistance to ischemia (12). The A_{2A} receptor may also play a role in protection from ischemia (34–36). In view of the recently proposed mechanism of action of CBD associating it with enhancement of adenosine signaling (5), one might speculate that the effect on the myocardium may be mediated via adenosine receptor effects.

In the past, corticosteroids were studied as a potential therapy for myocardial infarction because of their anti-inflammatory effect (10). However, this therapy has ultimately turned out to be harmful, inasmuch as corticosteroids delay and interfere with myocardial scar formation. This leads to thinner scars and increases susceptibility to rupture (30). In our experiments, we observed the treated animals for 7 days only. Thus it is possible that the beneficial effect observed after 7 days may become harmful over a longer time period. It might cause a delay in scar healing and also might reduce scar strength, leading to unfavorable LV remodeling in a manner similar to that of corticosteroids. Therefore, in CBD-treated animals, a longer period of follow-up is warranted to assess the long-term recovery and safety. However, we did not find any difference between the treated and control animals in relation to myofibroblast activity. This might suggest that CBD did not adversely affect the scarring process.

In our trial, we have shown that CBD induces a reduction in infarct size that is correlated with EF, is caused by reduced myocardial inflammation, and is not associated with favorable remodeling processes. We therefore assume that the reduced inflammatory response is mediated by an attenuated immunologic response. This is in agreement with other trials that have shown beneficial effects of CBD in a number of inflammatory diseases (2, 4, 6, 26, 33). However, we have yet to establish the precise inflammatory mechanism affected by CBD.

In conclusion, we have shown that CBD causes reduced infarct size in an in vivo rat model of ischemia and reperfusion. Furthermore, it seems that this effect is not direct and may be mediated by a reduced inflammatory response. Thus CBD may be a promising novel treatment for myocardial ischemia.

REFERENCES

1. **Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, Moriello AS, Davis JB, Mechoulam R, Di Marzo V.** Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR_1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol* 134: 845–852, 2001.
2. **Blake DR, Robson P, Ho M, Jubb RW, McCabe CS.** Preliminary assessment of the efficacy, tolerability and safety of a cannabis-based

medicine (Sativex) in the treatment of pain caused by rheumatoid arthritis. *Rheumatology* 45: 50–52, 2006.

3. **Bouchard JF, Lepicier P, Lamontagne D.** Contribution of endocannabinoids in the endothelial protection afforded by ischemic preconditioning in the isolated rat heart. *Life Sci* 72: 1859–1870, 2003.

4. **Brady CM, DasGupta R, Dalton C, Wiseman OJ, Berkley KJ, Fowler CJ.** An open-label pilot study of cannabis-based extracts for bladder dysfunction in advanced multiple sclerosis. *Mult Scler* 10: 425–433, 2004.

5. **Carrier EJ, Auchampach JA, Hillard CJ.** Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proc Natl Acad Sci USA* 103: 7895–7900, 2006.

6. **Croxford JL, Yamamura T.** Cannabinoids and the immune system: potential for the treatment of inflammatory diseases? *J Neuroimmunol* 166: 3–18, 2005.

7. **Cunha JM, Carlini EA, Pereira AE, Ramos OL, Pimentel C, Gagliardi R, Sanvito WL, Lander N, Mechoulam R.** Chronic administration of cannabidiol to healthy volunteers and epileptic patients. *Pharmacology* 21: 175–185, 1980.

8. **Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R.** Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258: 1946–1949, 1992.

9. **Gaoni Y, Mechoulam R.** The isolation and structure of delta-1-tetrahydrocannabinol and other neutral cannabinoids from hashish. *J Am Chem Soc* 93: 217–224, 1971.

10. **Giugliano GR, Giugliano RP, Gibson CM, Kuntz RE.** Meta-analysis of corticosteroid treatment in acute myocardial infarction. *Am J Cardiol* 91: 1055–1059, 2003.

11. **Headrick JP, Peart J.** A_3 adenosine receptor-mediated protection of the ischemic heart. Pharmacological preconditioning: potential new treatment modalities for the ischemic myocardium. *Vasc Pharmacol* 42: 271–279, 2005.

12. **Jacobson KA, Gao ZG.** Adenosine receptors as therapeutic targets. *Nat Rev Drug Discov* 5: 247–264, 2006.

13. **Joyeux M, Arnaud C, Godin-Ribuot D, Demenge P, Lamontagne D, Ribuot C.** Endocannabinoids are implicated in the infarct size-reducing effect conferred by heat stress preconditioning in isolated rat hearts. *Cardiovasc Res* 55: 619–625, 2002.

14. **Kitsis RN, Jialal I.** Limiting myocardial damage during acute myocardial infarction by inhibiting C-reactive protein. *N Engl J Med* 355: 513–515, 2006.

15. **Krylatov AV, Ugdyzhekova DS, Bernatskaya NA, Maslov LN, Mekhoulam R, Pertwee RG, Stephano GB.** Activation of type II cannabinoid receptors improves myocardial tolerance to arrhythmogenic effects of coronary occlusion and reperfusion. *Bull Exp Biol Med* 131: 523–525, 2001.

16. **Malfait AM, Gallily R, Sumariwalla PF, Malik AS, Andreakos E, Mechoulam R, Feldmann M.** The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. *Proc Natl Acad Sci USA* 97: 9561–9566, 2000.

17. **Mechoulam R.** *Cannabinoids as Therapeutics*, edited by Mechoulam R. Basel: Birkhauser Verlag, 2005.

18. **Mechoulam R, Hanus L.** Cannabidiol: an overview of some chemical and pharmacological aspects. I. Chemical aspects. *Chem Phys Lipids* 121: 35–43, 2002.

19. **Mirit E, Palmon A, Hasin Y, Horowitz M.** Heat acclimation induces changes in cardiac mechanical performance: the role of thyroid hormone. *Am J Physiol Regul Integr Comp Physiol* 276: R550–R558, 1999.

20. **Nachlas MM, Friedman MM, Cohen SP.** A method for the quantitation of myocardial infarcts and the relation of serum enzyme levels to infarct size. *Surgery* 55: 700–708, 1964.

21. **Nachlas MM, Shnitka TK.** Macroscopic identification of early myocardial infarcts by alterations in dehydrogenase activity. *Am J Pathol* 42: 379–405, 1963.

22. **Onaivi ES, Sugiura T, Di Marzo V.** *Endocannabinoids—The Brain and Body's Marijuana and Beyond.* London: Taylor and Francis, 2006.

23. **Pepys MB, Hirschfield GM, Tennent GA, Ruth Gallimore J, Kahan MC, Bellotti V, Hawkins PN, Myers RM, Smith MD, Polara A, Cobb AJA, Ley SV, Andrew Aquilina J, Robinson CV, Sharif I, Gray GA, Sabin CA, Jenvey MC, Kolstoe SE, Thompson D, Wood SP.** Targeting C-reactive protein for the treatment of cardiovascular disease. *Nature* 440: 1217–1221, 2006.

24. **Pertwee RG.** Pharmacological actions of cannabinoids. In: *Handbook of Experimental Pharmacology.* New York: Springer, 2005, p. 1–51.

25. **Pertwee RG, Thomas A, Stevenson LA, Maor Y, Mechoulam R.** Evidence that (−)-7-hydroxy-4′-dimethylheptyl-cannabidiol activates a non-CB_1, non-CB_2, non-TRPV1 target in the mouse vas deferens. *Neuropharmacology* 48: 1139–1146, 2005.

26. **Rog DJ, Nurmikko TJ, Friede T, Young CA.** Randomized, controlled trial of cannabis-based medicine in central pain in multiple sclerosis. *Neurology* 65: 812–819, 2005.

27. **Ugdyzhekova DS, Bernatskaya NA, Stefano JB, Graier VF, Tam SW, Mekhoulam R.** Endogenous cannabinoid anandamide increases heart resistance to arrhythmogenic effects of epinephrine: role of CB_1 and CB_2 receptors. *Bull Exp Biol Med* 131: 251–253, 2001.

28. **Ugdyzhekova DS, Krylatov AV, Bernatskaya NA, Maslov LN, Mechoulam R, Pertwee RG.** Activation of cannabinoid receptors decreases the area of ischemic myocardial necrosis. *Bull Exp Biol Med* 133: 125–126, 2002.

29. **Varga K, Lake K, Martin BR, Kunos G.** Novel antagonist implicates the CB_1 cannabinoid receptor in the hypotensive action of anandamide. *Eur J Pharmacol* 278: 279–283, 1995.

30. **Vivaldi MT, Eyre DR, Kloner RA, Schoen FJ.** Effects of methylprednisolone on collagen biosynthesis in healing acute myocardial infarction. *Am J Cardiol* 60: 424–425, 1987.

31. **Wagner JA, Hu K, Karcher J, Bauersachs J, Schafer A, Laser M, Han H, Ertl G.** CB_1 cannabinoid receptor antagonism promotes remodeling and cannabinoid treatment prevents endothelial dysfunction and hypotension in rats with myocardial infarction. *Br J Pharmacol* 138: 1251–1258, 2003.

32. **Wagner JA, Jarai Z, Batkai S, Kunos G.** Hemodynamic effects of cannabinoids: coronary and cerebral vasodilation mediated by cannabinoid CB_1 receptors. *Eur J Pharmacol* 423: 203–210, 2001.

33. **Weiss L, Zeira M, Reich S, Har-Noy M, Mechoulam R, Slavin S, Gallily R.** Cannabidiol lowers incidence of diabetes in non-obese diabetic mice. *Autoimmunity* 39: 143–151, 2006.

34. **Xu Z, Mueller RA, Park SS, Boysen PG, Cohen MV, Downey JM.** Cardioprotection with adenosine A_2 receptor activation at reperfusion. *J Cardiovasc Pharmacol* 46: 794–802, 2005.

35. **Yang Z, Day YJ, Toufektsian MC, Xu Y, Ramos SI, Marshall MA, French BA, Linden J.** Myocardial infarct-sparing effect of adenosine A_{2A} receptor activation is due to its action on CD4$^+$ T lymphocytes. *Circulation* 114: 2056–2064, 2006.

36. **Yang Z, Day YJ, Toufektsian MC, Ramos SI, Marshall M, Wang XQ, French BA, Linden J.** Infarct-sparing effect of A_{2A}-adenosine receptor activation is due primarily to its action on lymphocytes. *Circulation* 111: 2190–2197, 2005.

37. **Ytrehus K, Liu Y, Tsuchida A, Miura T, Liu GS, Yang XM, Herbert D, Cohen MV, Downey JM.** Rat and rabbit heart infarction: effects of anesthesia, perfusate, risk zone, and method of infarct sizing. *Am J Physiol Heart Circ Physiol* 267: H2383–H2390, 1994.

38. **Zajicek J, Fox P, Sanders H, Wright D, Vickery J, Nunn A, Thompson A.** Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo-controlled trial. *Lancet* 362: 1517–1526, 2003.