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European Journal of Pharmacology 469 (2003) 165–173
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Amiodarone minimizes experimental autoimmune myocarditis in rats

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Received 9 December 2002; received in revised form 13 March 2003; accepted 4 April 2003

Abstract

Amiodarone, a promising drug for the treatment of tachyarrythmias, was recently found to have immunomodulatory effects in vitro. We hypothesized that amiodarone would affect the immune system in vivo and examined the effect of amiodarone on myocarditis in rats. We induced experimental autoimmune myocarditis in rats by cardiac myosin immunization and treated the animals with an intraperitoneal injection of amiodarone at 25 mg/kg/every other day, 10 times after the induction of experimental autoimmune myocarditis. In the treated group, both microscopic and macroscopic examinations showed reduced heart weights, a mild and localized infiltration of inflammatory cells and fibrosis in the myocardium, and a mild congestion in the liver and lungs as compared with the control group. The phenotypic distribution of lymphocytes in peripheral blood showed a significant decrease in the CD4/CD8a ratio in the treated group, but not in the control group. The proportion of mast cells involved in inflammatory cell infiltration was lower in the treated group than the control group. In vitro, amiodarone inhibited the proliferation of mast cells by arresting them in the G2 phase of the cell cycle. These results indicated that amiodarone minimized the progression of experimental autoimmune myocarditis, suggesting a potential therapeutic role for amiodarone treatment in patients suffering from myocarditis, especially myocarditis complicated by cardiac arrhythmias. One possible mechanism by which amiodarone minimizes the progression of experimental autoimmune myocarditis may be to affect the immune system via the immunomodulatory effects on T cell and mast cell functions.

Keywords: Amiodarone; Myocarditis; Immune system; Lymphocyte; Mast cell

1. Introduction

Dilated cardiomyopathy is a multifaceted disease resulting from an insult to the ventricular myocardium and is characterized by a progressive enlargement of cardiac chambers, thinning of ventricular walls, and reduced contractility. Dilated cardiomyopathy is also a prevalent cause of heart failure and sudden death, and there is no specific therapy except for cardiac transplantation. Accumulating evidence links alcohol abuse, systemic hypertension, pregnancy, immunological disorders, viral infections, nutritional deficiency, and the effects of a number of chemical and physical agents, with the eventual development of dilated cardiomyopathy, although the exact etiology and pathogenesis of dilated cardiomyopathy remain to be defined (Wenger et al., 1990; Magnusson et al., 1994; Matsui et al., 1995; Chen et al., 20000). Recently, the autoimmune reactions of the host have been considered to be involved in the pathogenesis of myocarditis, similar to dilated cardiomyopathy (Goodwin, 1985; Matsui and Fu, 1998). Some cases of myocarditis are progressive, resulting in chronic myocarditis which may finally lead to dilated cardiomyopathy (Kodama et al., 1994; Seko et al., 1995).

Injection of porcine whole or rod cardiac myosin into susceptible strains of rats and mice has been shown to cause experimental autoimmune myocarditis (Neu et al., 1987; Wegmann et al., 1994; Inomata et al., 1995). Experimental autoimmune myocarditis has been demonstrated to be similar to the pathogenesis of some forms of human myocarditis

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0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01715-1

(Kodama et al., 1990; Kodama and Izumi, 1991) and was shown to occur in recurrent episodes by repeating the immunization with myosin in the recovery phase of inflammation, leading to dilated cardiomyopathy (Wilson et al., 1985; Kodama et al., 1994).

Amiodarone is an iodine-rich benzofuranic derivative frequently used in the management of a variety of life-threatening tachyarrhythmias including those, both ventricular and supraventricular, resistant to other drugs (Dorian et al., 2002; Madrid et al., 2002). Several lines of evidence suggest that the cell-mediated immune response is modulated by amiodarone (Akoun et al., 1988; Hostetler et al., 1991; Wilson et al., 1993; Matsumori et al., 1997). This prompted us to hypothesize that amiodarone interferes with the progression of autoimmune-mediated myocardial injury. Using experimental autoimmune myocarditis as a model, the present study was designed to study the effect of amiodarone on myocarditis.

2. Materials and methods

2.1. Reagents

The amiodarone used in this study was kindly donated by Sanofi-Synthelabo (Paris, France) (Fig. 1). The following monoclonal antibodies (mAbs) used for cytofluorometric analysis were purchased from Fujisawa Pharmaceutical (Osaka, Japan): *R*-phycoerythrin-conjugated mouse anti-rat CD45R (as a pan B cell marker) and CD8a (suppressor T cell marker) mAbs, and fluorescein isothiocyanate (FITC)-conjugated mouse anti-rat CD4 (helper T cell marker) mAb. The antibodies against rat mitotic phosphoprotein monoclonal-2 (MPM2, a hallmark of M phase), cyclin A (G2/M transition) and cyclin B (G2/M transition), used in this study were purchased from Wako (Tokyo, Japan). Mouse anti-rat CD4 mAb used for immunohistochemical analysis was purchased from Beckman Coulter (CA, USA). All other chemicals were of reagent grade and were purchased from Sigma (MO, USA).

2.2. Experimental animals

Specific pathogen-free male Lewis rats weighing 150 g were purchased from Charles River Japan. They were housed in animal quarters with controlled temperature (22–26 °C), humidity (50–60%), and lighting (12 h cycle)

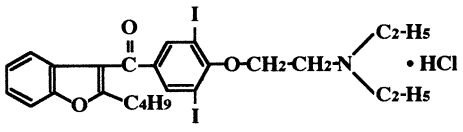

Fig. 1. Structural formula of amiodarone.

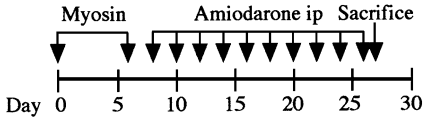

Fig. 2. Chart of experimental protocol.

and were given free access to standard feed and water. Body weights were measured at regular intervals.

2.3. Cell line and culture conditions

A mast cell line (RBL-2H3 cells, syngeneic to rats) (Turner et al., 1995) was grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 50 U/ml of penicillin, and 50 μg/ml of streptomycin. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. The viability of the cells used in these experiments was consistently more than 95% when evaluated by the trypan blue exclusion method.

2.4. Induction of experimental autoimmune myocarditis and administration of amiodarone

Purified cardiac myosin prepared from the muscles of porcine hearts and purchased from Sigma was used as an antigen. Experimental autoimmune myocarditis was induced as described by Inomata et al. (1995) with minor modifications. Briefly, the cardiac myosin was emulsified with complete Freund's adjuvant supplemented with Mycobacterium tuberculosis H37Ra at a concentration of 10 mg/ml. To induce myocarditis, Lewis rats were injected twice subcutaneously with a 7-day interval in the rear footpads with 0.1 ml of the emulsified solution. An intraperitoneal injection of amiodarone (designated the treated group, $n=10$) or an equal volume of saline as control (designated the control group, $n=10$) was administered to the animals at 25 mg/kg every other day, 10 times after the induction of experimental autoimmune myocarditis (Fig. 2).

2.5. Histopathologic analyses

For the histopathological examination of tissues, the rats were sacrificed under ether anesthesia on day 27 after the cardiac myosin injection. The heart, liver, lungs, thyroid, and pituitary were removed from the rats, weighed, sliced (hearts were cut transversely into at least four biventricular cardiac cross-sections), fixed in 10% formalin, embedded in paraffin, and stained. The extent of inflammatory cell infiltration was estimated using hematoxylin–eosin staining. The extent of fibrosis was estimated using Azan–Mallory staining. Mast cells were visualized by toluidine blue staining and counted.

Macroscopic findings of the heart were classified into five grades as described by Okura et al. (1998): 0, no

inflammation; 1, presence of a small discolored focus; 2, presence of multiple discolored foci; 3, diffuse discolored areas not exceeding a total of one third of the cardiac surface; and 4, diffuse discolored areas totaling more than one third of the cardiac surface.

Microscopic findings were expressed in terms of myocardial infiltration and fibrosis scores. Microscopic findings of the severity of myocardial infiltration and the extent of fibrosis were graded as follows (Okura et al., 1998): 0 (no lesion); 1 (presence of a few small lesions, not exceeding 0.25 mm^2 in size); 2 (presence of multiple small lesions or a few moderately sized lesions, not exceeding 6.25 mm^2); and 3 (presence of multiple moderately sized lesions or numerous larger lesions).

Microscopic scores represent the average, and mast cell numbers represent the total number in whole fields for all sections obtained from the same heart. Therefore, the microscopic scores and the mast cell numbers were defined as value/heart.

2.6. Immunohistochemical microscopy

The hearts, embedded in OCT compound, were frozen at −80 °C. Six-μm-thick frozen sections were cut in a cryostat and fixed in acetone for 5 min at 4 °C. Immunostaining of surface antigen CD4 in heart-infiltrating lymphocyte was performed according to the staining manual of the DAKO LSAB2 Kit (Dako, CA, USA) using mouse anti-rat CD4 mAb. The sections were observed using light microscopy.

2.7. Immunofluorescence lymphocyte subtyping

Peripheral blood was obtained from anesthetized rats by cardiac puncture. The blood was placed in a 1.2% EDTA solution in phosphate-buffered saline (PBS). The lymphocytes in 100 μl of blood were labeled with 20 μl of the fluorochrome-conjugated mouse anti-rat mAbs specific for lymphocyte surface antigens (CD4, CD8a, and CD45R). The lymphocytes were then incubated for 30 min and washed twice with PBS at 1500 rpm for 10 min. The tagged lymphocytes were fixed with 1% formaldehyde and stored at 4 °C until they were analyzed.

Immunofluorescence from individual cells was measured with a flow cytometer (Cytofluorograf system 50H, Ortho Instruments) and a FACSort (Becton Dickinson Immunocytometry System). At least 10^4 lymphocytes were acquired in each run, and the results were analyzed using CELLQuest software.

Table 1
Body and heart weights of experimental autoimmune myocarditis rats

Group	BW (g)	HW (mg)	HW (mg)/BW (g)
Normal group	328 ± 10[a]	1120 ± 103[b]	3.28 ± 0.28[c]
Control group	308 ± 19	1290 ± 74	3.89 ± 0.23
Treated group	299 ± 19	940 ± 70[c]	3.06 ± 0.27[c]

BW indicates body weight at the time of sacrifice, and HW indicates heart weight.
Values are expressed as the mean ± S.D. and are significantly different from those of the control group ([a]$P<0.05$, [b]$P<0.001$, [c]$P<0.0001$).

Table 2
Liver and lung weights of experimental autoimmune myocarditis rats

Group	LiW (mg)	LiW (mg)/BW (g)	LW (mg)	LW (mg)/BW (g)
Normal group	13150 ± 1370	38.3 ± 1.40	1160 ± 114[c]	3.38 ± 0.36[b]
Control group	13390 ± 1110	40.5 ± 3.78	1480 ± 84	4.46 ± 0.31
Treated group	11470 ± 1200[a]	36.6 ± 2.01[c]	1220 ± 45[c]	3.98 ± 0.15[c]

BW indicates body weight at the time of sacrifice, LiW indicates liver weight, and LW indicates lung weight.
Values are expressed as the mean ± S.D. and are significantly different from those of the control group ([a]$P<0.05$, [b]$P<0.001$, [c]$P<0.0001$).

2.8. Measurement of cellular proliferation and cell cycle analysis

The number of cultured mast cells was determined using a Coulter counter model ZM and a Channelyzer model 256 (Coulter Electronics). The analyzer was calibrated using 9.61-μm styrene beads.

The DNA content of the mast cells was determined as described previously (Zong et al., 1996). Briefly, the cells were fixed in 70% ethanol, treated exhaustively with pancreatic RNase A, and stained with propidium iodide (10 μg/ml in PBS). Fluorescence from individual cells was measured with a flow cytometer.

The MPM2, cyclin A, and cyclin B proteins of the mast cells were detected by indirect immunofluorescence using specific antibodies as described previously (Zong et al., 2000). Briefly, the mast cells were collected, washed in PBS, fixed with 1% formaldehyde for 30 min at room temperature, and treated with 0.3% Triton X-100 for 10 min at 37 °C. They were then incubated with the primary antibodies against MPM2, cyclin A, or cyclin B for 24 h at 4 °C. Next, they were washed in PBS containing 1% bovine serum albumin and stained with the secondary anti-immunoglobulin G FITC-conjugated antibodies. Finally, the cells were washed again, resuspended in PBS containing RNase A and propidium iodide, and analyzed by flow cytometry.

2.9. Assays of T3, T4, and TSH

Serum specimens were obtained at sacrifice and fractions were separated by centrifugation and stored at −80 °C until they were used. Triiodothyronine (T3) and thyroxine (T4) levels of rats were measured using an electrochemiluminescent immunoassay kit from Roche Diagnostics (Basel, Switzerland) (Blackburn et al., 1991) and thyroid stimulating hormone (TSH) levels, with a radioimmunoassay kit from Amersham Biosiences (NJ, USA) (Ishikawa et al., 1987). The detection limit of these assays was 0.195 ng/ml in T3, 0.42 μg/dl in T4, and 0.1 ng/ml in TSH.

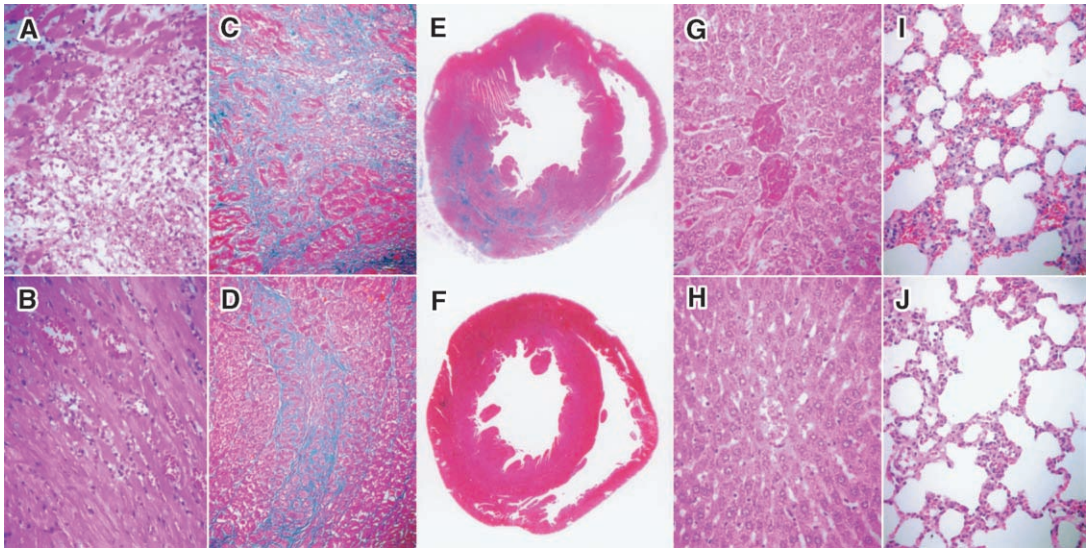

Fig. 3. Representative photomicrographs showing histopathological changes of the heart, liver and lung. Upper panel, the control group, and lower panel, the treated group, as described in "Materials and Methods". A, B, C, D, E, and F: heart; G and H: liver; I and J: lung. A, B, G, H, I, and J: hematoxylin–eosin staining, original magnification ×200. C and D: Azan–Mallory staining, original magnification ×100. E and F: Azan–Mallory staining, original magnification ×20.

2.10. Data analysis

All values were expressed as the mean ± S.D. Data were compared between the groups by using analysis of variance (ANOVA). A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Changes of body, heart, liver, and lung weights

In the normal group, the average body weight of the rats increased from 202 ± 3 g at the beginning to 328 ± 10 g at the end of the experiment, a gain of 126 g (Table 1). Weight gain was significantly lower in the control group at 100 g (208 to 308) and in the treated group at 97 g (202 to 299). Intraperitoneal injection of amiodarone did not significantly change the increase of body weight in the treated group, as compared with the control group. With regard to the heart, it was heavier in the control group (1290 ± 74 mg) and lighter in the treated group (940 ± 70 mg). When normalized to body weight at the time of sacrifice, there was a marked increase in the heart-to-body weight ratio in the control group (3.89 ± 0.23) compared with the treated group (3.06 ± 0.27). These results suggested that a more severe inflammation occurred in the hearts of the control group.

In addition to the findings in the heart, both the lung weight and the lung-to-body weight ratio were increased in the control group and decreased in the treated group (Table 2). Similar findings were observed in the liver. These results suggested that congestive heart failure occurred in the rats of the control group.

3.2. Histopathologic examination

To verify the prediction about heart disease in the rats, the heart, liver, and lungs were subjected to microscopic and macroscopic examinations (Fig. 3). In the control group, macroscopic examination revealed discoloration on the cardiac surface and slight adhesion of the cardiac surface to the parietal pericardium, while microscopic examination showed extensive myocardial necrosis, infiltration by inflammatory cells and myocardial fibrosis, as well as obvious congestion in the liver and lungs. Myocarditis

Table 3
Macroscopic and microscopic scores

Group	Macroscopic scores	Macroscopic scores
Control group	1.80 ± 0.42	2.40 ± 0.52
Treated group	0.60 ± 0.52[a]	0.90 ± 0.74[a]

Values are expressed as the mean ± S.D. and are significantly different from those of the control group ([a]$P < 0.01$).

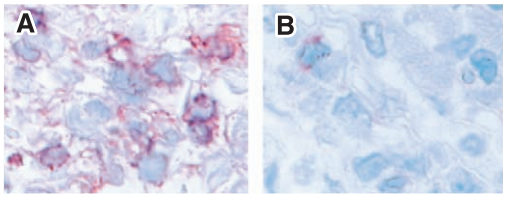

Fig. 4. Immunohistochemical microscopy of the heart-infiltrating cells. The hearts were removed from anesthetized rats in control group (A) and treated group (B) and stained with monoclonal antibody against surface antigen CD4 of heart-infiltrating lymphocyte as described in "Materials and Methods".

Table 4
Changes of lymphocyte subsets in peripheral blood

Group	CD4 (%)	CD8 (%)	CD45R (%)	CD4/CD8
Normal group	50.74 ± 3.07	18.24 ± 1.19	20.91 ± 3.11[b]	2.79 ± 0.25
Control group	57.90 ± 4.31	19.71 ± 1.69	16.70 ± 1.39	2.95 ± 0.24
Treated group	55.89 ± 3.65	22.26 ± 1.97	16.98 ± 2.38	2.51 ± 0.23[a]

Values are expressed as the mean ± S.D. and are significantly different from those of the control group ([a]$P<0.01$, [b]$P<0.001$).

and congestive heart failure, as a consequence of myocarditis, occurred in the rats of the control group.

In the treated group, the extent of inflammation and fibrosis in the heart and the congestion in the liver and lungs was markedly attenuated. The macroscopic and microscopic scores (hematoxylin–eosin staining) for heart were significantly lower in the treated group than the control group (Table 3).

The heart-infiltrating cells were investigated with immunohistochemical microscopy (Fig. 4). Many CD4-positive cells were observed in the control group, but not in the treated group. These results suggest that CD4-positive cells play an important role in the development of experimental autoimmune myocarditis.

3.3. Characterization of the phenotypes of lymphocytes in peripheral blood

To study the effect of amiodarone on the phenotypic distribution of lymphocytes in peripheral blood obtained under ether anesthesia by cardiac puncture at the end of the

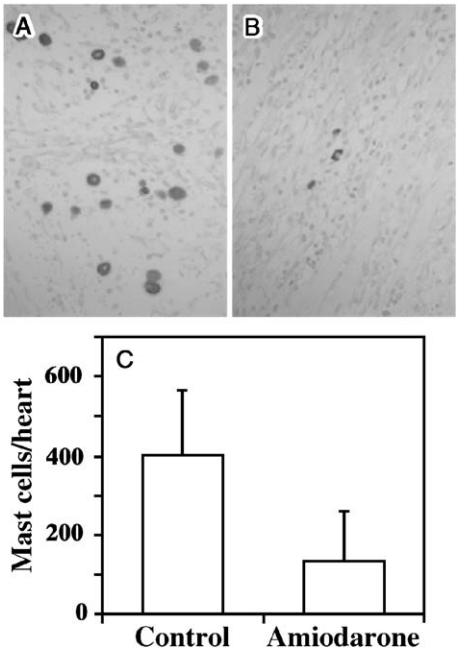

Fig. 5. Representative photomicrographs of mast cell accumulation in heart. (A) The control group and (B) the treated group as described in "Materials and Methods". (C) Mean value of mast cell accumulation in heart. Azan–Mallory staining, original magnification ×200.

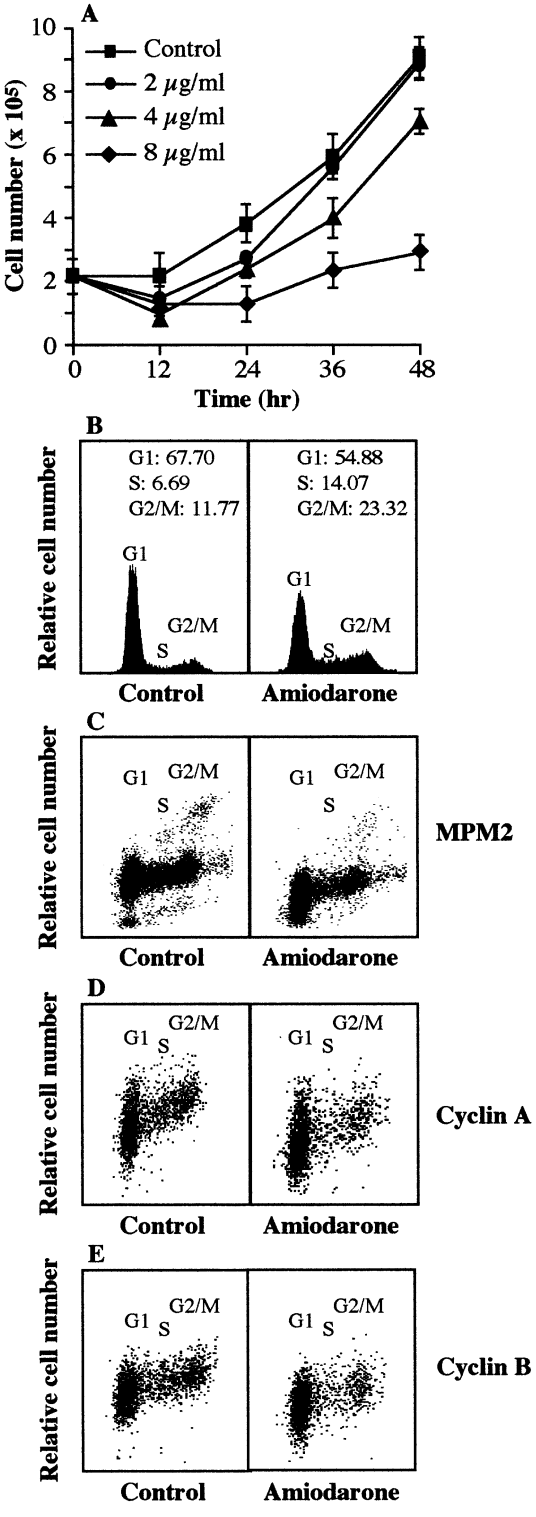

Fig. 6. Effects of amiodarone on cell growth, cell cycle and G2 checkpoint in mast cells. Mast cells were treated with 0, 2, 4, or 8 μg/ml of amiodarone for 48 h and then examined for cell numbers by a Coulter counter (A), distribution of DNA content (B), and expression of MPM2 (C), cyclin A (D) and cyclin B (E) proteins by flow cytometry as described in "Materials and Methods". Values are expressed as the mean ± S.D.

experiment, the phenotype was analyzed by flow cytometry. As compared with the normal group, the control group had a high proportion of CD4 cells and CD8a T cells, a high CD4/CD8a ratio, but a low proportion of CD45R cells; however, no significant difference was observed except for the CD45R cells ($P<0.001$) (Table 4). Compared with the control group, amiodarone administration resulted in an increase in the proportion of CD8a T cells, which led to a significant decrease in the CD4/CD8a ratio ($P<0.01$) in the treated group.

3.4. Effects of amiodarone on cell growth, cell cycle, and G2 checkpoint in mast cells

Corresponding with the intensity of inflammatory cell infiltration, we found in the area of the infiltration that there was a more marked accumulation of mast cells, visualized by toluidine blue staining, in the control group than the treated group (Fig. 5). This result implies that mast cells may play an important role in the progression of myocarditis.

To evaluate this possibility, we studied the effect of amiodarone on mast cells in vitro. For cell growth and cell cycle analyses, exponentially growing mast cells in non-synchronized cultures were treated with 0, 2, 4, or 8 μg/ml of amiodarone and then cultivated for 12, 24, 36, or 48 h. As shown in Fig. 6, cell counts show that the proliferation of mast cells was inhibited by amiodarone in a concentration-dependent manner (Fig. 6A), and the treatment with amiodarone increased the proportion of cells in the S and G2/M phases (Fig. 6B). These results suggest that amiodarone inhibits the proliferation of mast cells through G2/M arrest.

To clarify the behavior of cells in the G2 and M phases during the amiodarone-induced G2/M arrest, flow cytometry was used to quantify the expression of MPM2, cyclin A, and cyclin B proteins. Fig. 6C shows the expression of MPM2 protein in mast cells subjected to flow cytometry. After the exposure of cells to 8 μg/ml of amiodarone for 48 h, the expression of MPM2 protein was significantly inhibited, which suggests that amiodarone-treated cells did not enter M phase. Under the same conditions, the expression of both cyclin A (Fig. 6D) and cyclin B (Fig. 6E) proteins was also significantly inhibited. These results indicate that disruption to the G2 checkpoint is a causal factor of amiodarone-induced G2 arrest.

Table 5
Plasma levels of T3, T4, and TSH

Group	T3 (ng/ml)	T4 (μg/dl)	TSH (ng/ml)
Normal group	1.22 ± 0.08	7.42 ± 0.63[a]	7.08 ± 0.76
Control group	1.15 ± 0.12	5.65 ± 0.48	7.32 ± 0.73
Treated group	1.22 ± 0.07	7.02 ± 0.73[a]	7.12 ± 0.82

Values are expressed as the mean ± S.D. and are significantly different from those of the control group ([a]$P<0.001$).

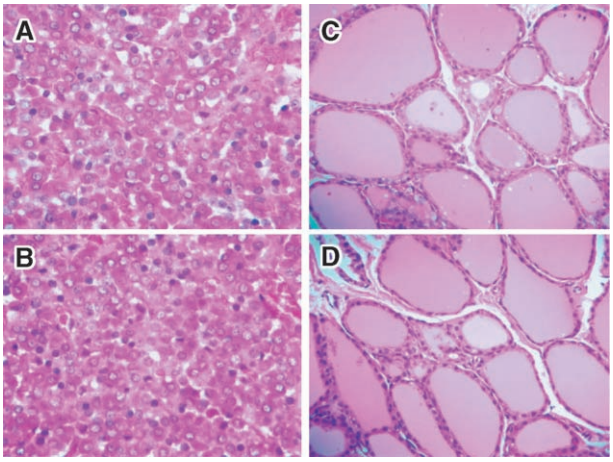

Fig. 7. Histopathologic examination of pituitary and thyroid. Upper panel, the control group, and lower panel, the treated group, as described in "Materials and Methods". A and B: pituitary; C and D: thyroid. Hematoxylin–eosin staining, original magnification ×200.

3.5. Effects of amiodarone on serum thyroid hormone levels

The mean data are shown in Table 5. In the control group, a trend toward a decrease in serum T3, an increase in TSH, and a significant decrease in T4 ($P<0.001$) were evident as compared with the normal group. A tendency of hypothyroidism was observed in the control group. Treatment with amiodarone for rats in the treated group normalized the tendency for hypothyroidism, and the mean data were nearly the same in the normal group and the treated group. Histopathologic examination of thyroid and pituitary did not reveal any abnormal change either in the control group or in the treated group (Fig. 7).

It is well established that amiodarone influences the level of serum thyroid hormones; it inhibits monodeiodination of T4 to T3, resulting in increased serum T4, free T4, reverse T3 concentrations, and decreased T3 concentration (Burger et al., 1976). The abovementioned results that amiodarone did not significantly increase serum T4 concentration and decrease T3 concentration suggest that amiodarone do not affect serum reverse T3 concentration.

4. Discussion

There are a few reports that amiodarone modulates immune responses. Wilson et al. (1993) described that alveolar macrophages isolated from amiodarone-fed rats were significantly less phagocytic, that the rats had significantly depressed delayed-type hypersensitivity responses, as well as that spleen cells isolated from the amiodarone-fed rats also had severely depressed mitogen responses. Matsumori et al. (1997) showed that amiodarone inhibits production of tumor necrosis factor-α by human mononuclear cells. Against this background, in the present study, we examined the effect of amiodarone on myocarditis in rats

using the experimental autoimmune myocarditis model. Rats in the control group showed histopathological alterations in the myocarditis including inflammatory cell infiltration involving numerous neutrophils, lymphocytes and macrophages, edema, myocardial necrosis, and fibrosis. Rats in the control group also showed marked congestion in the liver and lungs, a sign of congestive heart failure. However, in the treated group, rats showed only mild histopathological alterations in the myocarditis and no significant congestion in liver or lung. It is obvious that amiodarone interferes with the progression of experimental autoimmune myocarditis.

The precise mechanism(s) by which amiodarone disrupts the progression of experimental autoimmune myocarditis is not entirely clear, but several possible explanations should be considered. First, our observations revealed that in rats of the treated group, amiodarone did not significantly influence the phenotypic distribution of helper T cells (CD4+), suppressor T cells (CD8+), or B cells (CD45R) in the peripheral blood as compared with the rats of the control group. However, a mild decrease in the proportion of helper T cells and a mild increase in the proportion of suppressor T cells led to a significant decrease in the CD4/CD8a ratio. Studies have demonstrated that CD4+ T cells play a critical role in the pathogenesis of experimental autoimmune myocarditis (Neu et al., 1990; Smith and Allen, 1991) although multiple mechanisms probably cause the immune-mediated destruction of myocytes. Second, the infiltration of CD4+ T cells into the myocardium is an important step in the induction of myocarditis. Kodama and Izumi (1991) have demonstrated that immunization with cardiac myosin activated myosin-reactive CD4+ T cells, as shown by the fact that most cells that infiltrated the heart were macrophages and CD4+ T cells. In the present study, we showed that many CD4-positive heart-infiltrating cells were observed in the control group, but not in the treated group. These results strongly supported this assumption. Similar to this study, we previously demonstrated that many such cells were present in the experimental autoimmune myocarditis rats, but not the treated rats (Song et al., 2001). Third, cytokines have been documented to play a crucial role in the pathogenesis of myocarditis. In animals with experimental autoimmune myocarditis, levels of tumor necrosis factor-α, interleukin-1β, and interleukin-6 have been found to be elevated in serum, heart tissue, and pericardial effusion (Nakayama et al., 2000; Afanasyeva et al., 2001). In human mononuclear cells, production of tumor necrosis factor-α has been found to be inhibited by amiodarone (Matsumori et al., 1997). These results strongly supported the possibility that amiodarone interferes with the progression of experimental autoimmune myocarditis by inhibiting the production of cytokines. We did not observe the effects of amiodarone on cytokines; therefore, additional studies are necessary using lymphocytes from experimental animals and patients with myocarditis. Fourth, mast cells are multifunctional cells that contain various mediators such as cytokines, histamine, proteases, and leukotrienes, and play a role in many types of inflammations, repair processes, and immunological reactions (Galli and Wirshel, 1995; Marone et al., 1995; Church and Levi-Schffer, 1997; Welle, 1997). Mast cells are present in almost all the major organs of the body and even more abundant in human heart tissue following heart failure, myocarditis, or dilated cardiomyopathy (Dvorak, 1986; Engels et al., 1995; Patella et al., 1998). In the present study, we found in the area of inflammatory cell infiltration, corresponding to the intensity of the infiltration, that there was a more marked accumulation of mast cells in the control group than the treated group. Likewise, in an in vitro experiment, we found that the proliferation of mast cells was significantly inhibited by amiodarone and that downregulation of a G2 checkpoint-related protein is a causal factor in arresting the cells in G2 phase. These results indirectly suggest that the effect of amiodarone on mast cells may affect the progression of myocarditis and dilated cardiomyopathy. An attempt to investigate the amiodarone-induced functional changes in mast cells and the role of mast cells in the progression of myocarditis, e.g., the effect of amiodarone on degranulation of mast cells and the effect that depletes mast cells from the rats on the progression of myocarditis, is underway. Last, amiodarone treatment can cause thyroid dysfunction including both thyrotoxicosis and hypothyroidism (Osman et al., 2002). Increasing evidence shows that thyroid status affects immune response (Fabris, 1973; Pacini et al., 1983; Fabris et al., 1995). Weiss and Davies (1981) reported that antithyroid drugs, such as methimazole and propylthiouracil, inhibit immunoglobulin-secreting cells. In a previous report, we showed that methimazole interferes with the progression of myocarditis in rats (Song et al., 2001). Therefore, the possibility that amiodarone minimizes the progression of myocarditis by disturbing the function of thyroid, should be considered. To test this possibility, we determined the concentrations of T3, T4, and TSH in serum specimens of peripheral blood and histopathologically examined the thyroid and pituitary. We found a tendency for hypothyroidism in the control rats and that treatment with amiodarone normalized the tendency. The state of myocarditis and treatment with amiodarone did not induce morphological changes in the thyroid and the pituitary. These results suggest that amiodarone could not affect the function of the thyroid in the myocarditis rats and normalized the tendency for hypothyroidism by minimizing the progression of myocarditis. Therefore, a strong possibility that inhibitory effect of amiodarone on myocarditis does not result from hypothyroidism, but from a direct effect on the immune system.

Although the precise mechanisms involved are largely unknown, it is a fact that amiodarone treatment in rats suffering from experimental autoimmune myocarditis effectively minimizes the progression of myocarditis. Thus, amiodarone may be a useful agent for the treatment of autoimmune-mediated myocardial injury. One possible

mechanism by which amiodarone minimizes the progression of experimental autoimmune myocarditis may be to affect the immune system via the immunomodulatory effects on T cell and mast cell functions. We anticipate the administration of amiodarone to be helpful in pharmacological therapy for autoimmune myocarditis and the prevention of postmyocarditis dilated cardiomyopathy. Undoubtedly, caution, e.g., about the dose of amiodarone used in this study, is required when extrapolating these results to patients with autoimmune-mediated myocarditis, and more studies on human myocarditis are necessary to determine whether these findings can be translated to the clinical setting.

Acknowledgements

This study was supported in part by a Grant for Project Research from High-Technology Center of Kanazawa Medical University (H2002-8).

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