Early Myocardial Lesions Induced by Cardiotoxic Compounds in Sprague-Dawley Rats

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ABSTRACT. Early focal myocardial lesions in rats induced by five cardiotoxic compounds were histopathologically observed 1 hr and 4 hr after a single intravenous injection with 1/10 LD₅₀ and LD₅₀. The lesions were observed 1 hr and 4 hr after the treatment with LD₅₀ of isoproterenol (ISP), 4 hr with 1/10 LD₅₀ of ISP, 4 hr with LD₅₀ of hydralazine (HYD), caffeine (CAF) and cyclophosphamide (CYC), but not with adriamycin (ADR). The lesions consisted of homogeneously intensely eosinophilic staining, contraction band formation and fragmentation of cardiac muscle fibers. The lesions were interspersed in the inner one third of the left ventricular walls including the papillary muscles with ISP, HYD and CAF, and were all over the ventricular myocardium with CYC. — **KEY WORDS:** cardiotoxic compound, histopathology, rat heart.

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Medicinal compounds with various pharmacological activities have been reported to induce myocardial lesions in rats [2–10, 12, 13]. Histological examinations are also often undertaken several weeks or months after treatment. The histological lesions often described are healing processes, and are mainly characterized by fibrosis. In routine toxicological studies in rats for safety assessment on new compounds, it is difficult to detect early myocardial lesions after a single or repeated treatments. It is important to determine early myocardial lesions in the rat heart after standardization of procedures to form morphological basis on which routine evaluation of myocardial lesions can be undertaken. In previously published reports on compound-induced myocardial lesions, the results might be difficult to provide references for evaluation of myocardial lesions observed in safety assessment of new compounds because the results varied due to the differences in age and strain of animals, route of administration, and time of examination after treatment.

In this study, therefore, five compounds which have been known to induce cardiotoxic disturbances in rats at very high doses are selected to evaluate early myocardial lesions.

One hundred forty male Sprague-Dawley rats [SPF/VAF

Crj: CD (SD)] were purchased from Charles River Japan, Atsugi Breeding Center, for the experiment. The animals were approximately 10 weeks of age and weighed approximately 250 g at the initiation of the experiment, and were individually housed in a meshed stainless cage in a climate-controlled animal room (temperature: 22 ± 2°C, relative humidity: 55 ± 5%, and lighting period: 7:00 AM to 7:00 PM). The animals were fed available food (Pelleted rodent chow, CR-1, Clea Japan Inc.) and tap water *ad libitum*.

Animal groups of 5 rats with 1/10 LD₅₀ dose and of 20 rats with LD₅₀ dose each were administered five compounds (Sigma Chemical Co.) as described below (Table 1): 1 and 10 mg/kg of adriamycin HCl (ADR), an anthracycline anticancer agent; 10 and 100 mg/kg of caffeine HCl (CAF), a vasoconstrictor at a very high dose; 16 and 160 mg/kg of cyclophosphamide HCl (CYC), an anticancer agent with thrombogenic potential; 3.4 and 34 mg/kg of hydralazine HCl (HYD), a vasodilating antihypertensive compound; and 1 and 10 mg/kg of isoproterenol HCl (ISP), a vasodilating antihypertensive compound with a β adreno-receptor stimulation. The compounds were dissolved in saline for injection (Ohtsuka Pharmaceutical Co.), and were

Table 1. Treatment groups and occurrence of rats with myocardial lesions

Compound group	Dose level		No. of rats with lesions posttreatment	
	mg/kg	Value	1 hr	4 hr
Saline (control)	—	—	0/5	0/10
Isoproterenol HCl (ISP)	1	1/10 LD ₅₀	n.t.	4/5
	10	LD ₅₀	10/10	10/10
Hydralazine HCl (HYD)	3.4	1/10 LD ₅₀	n.t.	0/5
	34	LD ₅₀	0/10	10/10
Caffeine HCl (CAF)	10	1/10 LD ₅₀	n.t.	0/5
	100	LD ₅₀	0/10	7/10
Cyclophosphamide HCl (CYC)	16	1/10 LD ₅₀	n.t.	0/5
	160	LD ₅₀	0/10	8/10
Adriamycin HCl (ADR)	1	1/10 LD ₅₀	n.t.	0/5
	10	LD ₅₀	0/10	0/10

n.t.=not tested

administered intravenously at 2 ml/kg *via* the tail vein. Either 5 or 10 rats each were administered only saline and served as control groups.

The animals were killed at 1 hr (10 rats with LD₅₀) and 4 hr (10 rats with LD₅₀ and 5 rats with 1/10 LD₅₀) after treatment by exsanguination from the thoracic vena cava under ether anesthesia, and the hearts were fixed in 10% neutrally buffered formalin immediately after death. Thereafter, the hearts were trimmed and sectioned longitudinally. The sections included all areas from the base to apex, as included the both ventricular walls, septum and papillary muscles. The hearts from 4 of the 5 rats and 9 of the 10 rats with 1/10 LD₅₀ and LD₅₀ each were processed into 4 μ m thick paraffin sections and stained with hematoxylin and eosin (HE), periodic acid Schiff (PAS) reaction, phosphotungstic acid hematoxylin (PTAH) and Masson's trichrome stains. In addition, the heart from one remaining animal from each animal group was embedded in methacrylate resin (JB-4 Embedding Kit TM, Polysciences, Inc., U.S.A.), and the sections cut at 1 μ m thickness were stained with HE and Watanabe's silver stains to examine the relationship between myocardial lesions and vascular alterations.

Early myocardial lesions were observed in the group 1 hr after the ISP treatment with LD₅₀ dose and 4 hr after the ISP, HYD, CAF and CYC with LD₅₀. There were no early myocardial lesions in the CAF, HYD and CYC at 1 hr after treatment with LD₅₀ and in the ADR of 1 hr and 4 hr with LD₅₀. Out of the groups treated with the 1/10 LD₅₀, only the 4 hr group of the ISP developed similar myocardial lesions.

In all the 10 rats of the group observed 1 hr after the ISP treatment with a dose of LD₅₀ (10 mg/kg), a few focal myocardial lesions were observed in the inner one third of the left ventricular walls including the papillary muscles at the apex of the hearts (Table 1), and the individual focal lesions consisted of two to five cardiac muscle fibers showing homogeneously intensely eosinophilic staining. The lesions were not accompanied by inflammatory response

nor hemorrhages (Fig. 1).

In the 4 hr group treated with LD₅₀ ISP, focal myocardial lesions were prominent in all the 10 animals. The focal lesions distributed more extensively in the inner one third of the left and right ventricular walls and septum (Fig. 5), and the individual focal lesions were larger and consisted of five to ten affected muscle fibers without hemorrhages. There were no proliferations of fibroblasts or fibrocytes. HE stain with the methacrylate resin-embedded sections revealed occasional mononuclear cell infiltrations into the larger lesions. The affected muscle fibers were shown to have intensely eosinophilic cytoplasm, formation of contraction bands, and fragmentation in some muscle fibers with or without margination of nuclei. The contraction bands and fragmentation were clearly demonstrated by Masson's trichrome and PTAH stains (Fig. 2). Silver stain with the methacrylate resin-embedded sections also showed formation of contraction bands and loss of striations in the affected muscle fibers. There were no hemorrhages, and the microvasculature remained intact (Fig. 3). The severity and distribution of the lesions increased in relation to increasing dose of ISP.

In the HYD- and CAF-treated groups, a few focal myocardial lesions similar to those in the ISP were observed in the inner one third of the left ventricular walls including papillary muscles at the apex of the hearts in rats 4 hr after treatment with both the doses of LD₅₀ of HYD and CAF (34 and 100 mg/kg, respectively). The lesions were also observed in the left side of the ventricular septum in the HYD group. There were lesions in all the 10 rats of the HYD group and 7 of the 10 rats of the CAF. No myocardial lesions were found at 1 hr after treatment of HYD and CAF with LD₅₀. The individual focal lesions in 4 hr groups of HYD and CAF with LD₅₀ were smaller than those observed in 4 hr group of the ISP, and consisted of two to five homogeneously intensely eosinophilic muscle fibers without cellular infiltration. A few affected muscle fibers showed formation of contraction bands and fragmentation, which were associated with or without margination of nuclei.

Fig. 1. The cardiac muscle fibers with contraction band formation without cell response in the inner one third of the left ventricular walls at 1 hr after treatment with 10 mg/kg of ISP. HE, $\times 190$.

Fig. 2. The cardiac muscle fibers with contraction band formation and fragmented cytoplasm, and infiltration of mononuclear cells in the inner one third of the left ventricular walls at 4 hr after the treatment with 10 mg/kg of ISP. PTAH, $\times 190$.

Fig. 3. Formation of contraction bands and loss of the striation in the affected cardiac muscle fibers without alterations of microvasculature in the inner one third of the left ventricular walls at 4 hr after treatment with 10 mg/kg of ISP. Silver of methacrylate-embedded section, $\times 190$.

Fig. 4. Cardiac muscle fibers with the fragmented cytoplasm and a small hemorrhage in the inner one third of the left ventricular walls at 4 hr after treatment with 100 mg/kg of CAF. PTAH, $\times 190$.

Fig. 5. Distribution of ISP-, HYD- and CAF-induced early focal myocardial lesions in rats 4 hr after a single LD_{50} treatment.

There were no hemorrhages, and the microvasculature remained intact in the HYD group. In the CAF group, the lesions were accompanied with focal hemorrhages (Fig. 4). No alterations of the microvasculature were found histomorphologically in the CAF group even in the methacrylate resin-embedded silver-stained sections. There were no morphological changes in both the myocardium and microvasculature at 4 hr after treatments with the doses of $1/10 LD_{50}$ of HYD and CAF (3.4 and 10 mg/kg, respectively).

There were a few very small focal myocardial lesions in the CYC group with the dose of LD_{50} (160 mg/kg), and the lesions were scattered in all over the ventricular walls including the left, right and septal walls, and the papillary muscles in the hearts of 8 of the 10 rats at 4 hr after the treatment. The individual focal lesions consisted of one to three homogeneously intensely eosinophilic cardiac muscle fibers, and were accompanied with focal hemorrhages similar to those in the groups of CAF with LD_{50} (Fig. 4), and with no cellular response. Masson's trichrome and PTAH stains revealed formation of contraction bands in some affected cells. The affection of cardiac muscle cells were very similar to those in the groups of ISP, HYD and CAF. No alterations of the microvasculature were found even in the methacrylate resin-embedded sections with the

silver stain. There were no myocardial lesions in the rats with the dose of $1/10 LD_{50}$ (16 mg/kg) of CYC at 4 hr after treatment.

There were no myocardial changes in the hearts from all the animals ADR-treated with the doses of $1/10 LD_{50}$ at 4 hr and LD_{50} (10 mg/kg) at both 1 hr and 4 hr after treatment.

There were no myocardial changes in the hearts from all the control animals at 1 hr and 4 hr after treatment with saline.

Histopathology of the compound-induced focal myocardial lesions at 4 hr, which were characterized by homogeneously intensely eosinophilic staining of cardiac muscle cells in the inner one third of the left ventricular walls, papillary muscles and/or septum at the apex, were similar among the ISP, HYD and CAF groups at the doses of LD_{50} (Fig. 5). These heart regions at which the myocardial lesions were observed are known to be most sensitive to hypoxic or ischemic conditions in the heart [1, 11]. Based on the distribution of the present focal myocardial lesions with or without hemorrhages, pharmacologically-mediated regional hypoxia or ischemia under increased workload in the myocardium might have been involved in pathogenesis of the lesions with these three compounds.

The CYC-induced lesions were interspersed all over the heart, and were accompanied with focal hemorrhages. CYC has been known to induce microvascular alterations, such as endothelial swelling and microthrombosis but not constrictive effect on the vessels, with very high dose in experimental animals [5, 7]. The present myocardial lesions might have been related to restricted hypoxia or ischemia to the very small heart regions due to the microvascular circulation, based on the distribution.

ADR has been known to cause myocardial hypertrophy in experimental animals a few weeks after treatment [9].

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