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Enhanced myocardial necrosis induced in rats by the combined administration of hydralazine and prenalterol

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The cardiotoxic effects of hydralazine and prenalterol, given alone and in combination, were assessed in rats and rabbits. Acute myocardial necrosis was induced by a single administration of each drug alone in rats. However, the incidence and severity of lesions were markedly enhanced when both drugs were given in combination. Rats that received the same treatment for 10 consecutive days showed minimal or no acute necrosis, demonstrating the development of a resistance to further cardiotoxic effects of the drugs. Rabbits showed only minimal lesions when either drug was used alone and no enhancement of lesions when they were given in combination. From these data, it is concluded that the possibility of a cardiotoxic interaction exists when these drugs are used in combination and that the heavy rat (500–600 g) is a more sensitive model than the rabbit for studies of this nature.

The combination of vasodilator and positive inotropic drugs is used in the therapy of congestive cardiac failure (Mikulic et al 1977; Cohn & Franciosa 1978; Drexler et al 1981). These two classes of drugs act by independent mechanisms in improving left ventricular pump function. Beneficial haemodynamic effects, viz. increased cardiac output, stroke volume and stroke work index, with a concomitant decrease in peripheral resistance, are markedly enhanced by the simultaneous administration of these drugs. However, this combined use could produce adverse cardiac effects including an increased incidence of ventricular premature beats, shortness of breath and palpitations (Drexler et al 1981).

These adverse effects can be attributed to myocardial hypoxia brought about by exaggerated pharmacological effects, i.e. hypotension and tachycardia. Large doses of vasodilating antihypertensive drugs and β-adrenoceptor agonists were shown to produce myocardial necrosis in laboratory animals as a result of these effects (Balazs & Bloom 1982). This development of lesions serves as a model for the cardiotoxicity of these drugs. By this means we tested representatives of the above classes of drugs, viz. hydralazine and prenalterol, when given alone and in combination for their cardiotonic effects in heavy rats and rabbits.

Methods

Thirty-two male Sprague-Dawley rats (4 to 5 months old) 500–600 g (heavy) were assigned to four groups of eight. The first three groups were each given a single injection, in saline, of hydralazine (10 mg kg^{-1} s.c.), prenalterol (2 mg kg^{-1} s.c.) or a combination of these drugs (hydralazine being given 20 min before prenalterol). Rats of the fourth group received saline. An additional four groups of rats, as described above, received the same treatment for 10 consecutive days. All rats were killed 48 h after treatment.

In another experiment, 40 New Zealand white male rabbits (3–4 kg) were divided into 8 groups. Animals of the first group were given hydralazine 10 mg kg^{-1} s.c.; the second, third and fourth groups received prenalterol 0·3, 1 and 3 mg kg^{-1} s.c., respectively, and the next three groups received a combination of these drugs (hydralazine being given 20 min before prenalterol) at each of the above doses. The last group of rabbits received saline. All treatments were given for two consecutive days and the rabbits were killed 48 h after the second dose.

Hearts from all animals were examined in-situ for gross lesions. They were fixed in 4% buffered formaldehyde and processed by routine histological procedures.

Sections were cut at apical and mid-ventricular levels from rat hearts and from the atria, ventricles, papillary muscles and apex from rabbit hearts. They were stained by H & E or Masson's trichrome method. Slides were examined by an investigator who had no knowledge of the treatment given to various groups of animals. Each section was judged on a scale of 0 to 4 according to the extent of the lesion: 0, no recognizable necrosis; 1, minimal (<5%); 2, slight (5–10%); 3, moderate (10–15%); 4, severe (>15%). Data were analysed by Wilcoxon rank-sum statistics.

Results

All rats survived the treatments. Gross examination of hearts revealed no lesions. Histopathologically, varying degrees of myocardial lesions were observed in rats that received different treatments. Lesions were mainly seen at the apex, left ventricular walls and papillary muscles and were localized predominantly in the subendocardial layer of the free wall and septum of the left ventricle. Fresh lesions consisted of small foci or large areas of necrosis associated with interstitial oedema and inflammatory cell infiltration. The necrotic muscle cells showed a loss of the normal pattern of cross striations. The incidence and degree of myocardial lesions in rats treated with single or multiple doses of hydralazine or prenalterol alone and in combination are summarized in Table 1. A single dose of hydralazine at 10 mg kg^{-1} produced acute necrosis in 1 of the 8 rats with an average lesion score of 0·4. Single doses of prenalterol alone produced acute lesions in 2 of the 8 rats with a mean lesion score of 0·9. When single doses of these

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Table 1. Incidence and degree of myocardial lesions in heavy rats (500–600 g) treated with a single or multiple doses (10 consecutive days) of hydralazine or prenalterol alone and in combination.

Treatment (mg kg^{-1} s.c.)	Single dose		Multiple doses	
	No. of rats with lesion score >2	Mean lesion grade[a]	Mean lesion grade[a]	
		Acute necrosis	Acute necrosis	Scar
Saline	0/8	0	0	0·5 (0–1·0)
Hydralazine (10)	1/8	0·4 (0–1·5)	0	1·7 (1·0–2·5)
Prenalterol (2)	2/8	0·9 (0·5–2·0)	0·2 (0–0·5)	0·8 (0·5–2·0)
Hydralazine[b] (10) + prenalterol (2)	7/8	2·5[c] (0·5–4·0)	0·2 (0–0·5)	1·6 (0·5–3·5)

[a] Each value, with its range in parentheses, represents the mean lesion grade from 8 hearts. Lesions were graded on a scale of 0–4. 0 = absent, 1 = minimal, 2 = slight, 3 = moderate, 4 = severe.
[b] Hydralazine was given 20 min before prenalterol administration.
[c] Significantly different ($P < 0·01$) from values of groups given hydralazine or prenalterol alone.

drugs were given in combination, 7 of the 8 rats showed acute myocardial necrosis; moreover, the severity of lesions was significantly increased, with an average lesion score of 2·5. Rats that received hydralazine or prenalterol alone or in combination for 10 consecutive days showed minimal or no acute necrosis; however, all of them had varying degrees of healed lesions (scars). These lesions consisted of collagen and fibroblasts with or without chronic inflammatory cells. The saline-treated rats did not have lesions.

All rabbits also survived the treatments, and gross examination of hearts showed no lesions. Microscopically, rabbits treated with hydralazine or graded doses of prenalterol alone produced only minimal myocardial lesions that were not significantly different from those seen in controls. Moreover, no enhancement of lesions was observed when these drugs were given in combination.

Discussion

The results of the present study indicate that the incidence and severity of acute myocardial necrosis were greatest when hydralazine and prenalterol were given in combination. This effect was observed only in heavy rats; no such enhancement was observed in rabbits. Balazs et al (1962) reported that rats weighing 400–500 g are more sensitive to the cardiotoxicity of isoprenaline than rats of 150–200 g body weight. We have previously shown that mortality, arrhythmia and myocardial lesion-inducing effects of isoprenaline were significantly enhanced by aminophylline in heavy rats and suggested that the heavy rat might be a sensitive model for studying cardiac interaction of such drug combinations (Joseph et al 1981). Whitehurst et al (1983) also reported that the cardiotoxic effects of terbutaline in combination with aminophylline were more marked in heavy rats and that no such interaction was seen in rabbits. Data from the present study further confirmed the usefulness of the heavy rat as a suitable model for studies of this nature.

The lack of development of fresh myocardial necrosis in rats treated for 10 consecutive days with each drug alone or in combination demonstrates the development of resistance to further necrogenic effects of the agents. Such a development of resistance in rats, which had been previously reported for isoprenaline (Balazs et al 1972; Joseph et al 1983), may be attributable to an adaptive alteration of the myocardium.

Although prenalterol is beneficial in the therapy of congestive heart failure (Tweddel et al 1980; Doering et al 1982), it is reported to have certain arrhythmogenic properties (Leinberger et al 1982). Hydralazine, which is also used in the treatment of chronic congestive heart failure (Chatterjee et al 1976; Rubin et al 1979), may provoke myocardial ischaemia with or without reflex tachycardia (Packer et al 1978) and may even produce myocardial infarction in man (Nickerson 1965). It is conceivable that a β-adrenoceptor agonist like prenalterol aggravates the adverse cardiac effects of hydralazine because its positive inotropic and chronotropic effects are superimposed on the hydralazine-induced hypotension and reflex tachycardia, thereby increasing the oxygen demand when the supply is reduced. Consequently, myocardial hypoxia develops, particularly in the least perfused subendocardial portion of the left ventricle. These events, along with other factors such as cyclic AMP activity, altered membrane permeability, excessive intracellular calcium accumulation etc, could be responsible for the development of myocardial lesions. Considering the different sensitivities of rats and rabbits, the results of our studies cannot be directly extrapolated to man; nevertheless the possibility of a cardiotoxic interaction should not be overlooked in the clinical use of these two classes of drugs in combination.

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Optimizing the pentetrazol infusion test for seizure threshold measurement

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Seizure thresholds in mice were determined using the pentetrazol infusion method. Concentration of infusate and rate of infusion were varied to assess the optimal parameters for seizure threshold detection. Seizure threshold elevations were produced by flurazepam and threshold decreases by FG 7142. An infusion rate of 1·1 ml min^{-1} was best for detecting both increases and decreases in threshold. However a concentration of 2·5 mg ml^{-1} gave optimal measurement of elevations in threshold whereas decreased thresholds were best detected with a concentration of 10 mg ml^{-1}.

Pentetrazol (leptazol, metrazol) is probably the most widely used convulsant for evaluating both the anticonvulsant and proconvulsant effects of drugs. After its initial introduction into clinical practice as a substitute for camphor in the therapeutic production of seizures (Meduna & Friedman 1939) it was widely used in animal experiments (see Hahn 1960; Stone 1972). For many years the 'metrazol' test which used either i.p. or s.c. pentetrazol to produce seizures has been the primary screen for anticonvulsant activity in new pharmaceutical compounds. In addition, as the benzodiazepines were so effective against pentetrazol effects, and the relative potency of these effects followed closely their clinical dosage (Randall & Kappell 1973), the test was also used to screen for novel anti-anxiety compounds. Unfortunately the i.p. or s.c. routes of administration pose problems when pentetrazol is used in a more precise or quantitative fashion than is generally the case with screening tests. For instance, we have shown that the variability of both seizure latency and incidence with i.p. pentetrazol made it difficult to use in evaluating the effects of electroconvulsive treatment on seizure susceptibility in rats (Nutt et al 1980). In preference we developed an intravenous infusion method similar to that used previously by others (Orloff et al 1949; Chen et al 1954; Hint & Richter 1958). This gave consistent and reliable values of seizure threshold, and was able to detect drug effects using many fewer animals than the earlier ED50 or CD50 tests (see Stone 1972). We have now calibrated the i.v. pentetrazol infusion in mice in an attempt to determine the optimal conditions of drug concentration and infusion rate for the detection of both elevations and reductions in seizure susceptibility.

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Methods

Male Charles River CD_1 mice (30–35 g) were used. Pentetrazol was dissolved in saline at concentrations of either 1, 2·5 or 10 mg ml^{-1} and infused at different rates. Infusion rates used ranged from 0·138 to 1·1 ml min^{-1}. Seizure threshold elevations (anticonvulsant effects) were produced by pretreatment with the water-soluble benzodiazepine, flurazepam, 10 mg kg^{-1} given i.p. (10 ml kg^{-1} in saline). Seizure threshold reductions (proconvulsant effects) were produced by treatment with the β-carboline benzodiazepine receptor ligand FG 1742 (ethyl β-carboline-3-carboxylate methylamide; see