# PRODUCTION OF MYOCARDIAL LESIONS IN A GRADED FASHION BY NONINVASIVE TECHNIQUE IN ALBINO RATS

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**Summary :** Systematic study of producing graded lesions of myocardium with the use of Iscproterenol (IPT) reveals that mild to severe degree of infarction can be induced by administering the compound for one to four consecutive days in the dose of 85 *mg/kg* body weight in rats. These findings differ slightly from those of Rona *et al*. where they have not attempted to produce the lesions in a graded fashion. The experimental period can be reduced to two days by using high dose (170 *mg/kg*), but such procedure increases significantly the mortality in these animals and lesions are not produced in graded fashion.

Graded lesions of myocardium are closely related to electrocardiographic changes and serum CPK levels.

The study emphasizes the importance of the number of doses of IPT given at twenty four hour intervals for inducing a lesion in graded fashion. Drugs having weaker potential activity can even be evaluated with the help of such graded lesions.

**Key words :** myocardial lesions isoproterenol

## INTRODUCTION

Administration of isoproterenol (IPT) to produce myocardial lession is considered to be an important non–invasive experimental technique in rats. Clinically, the quantity of infarcted myocardium determines the prognosis in patients (8, 11, 16). Production of myocardial lesion for pharmacological intervention has aroused considerable interest in this field (8, 11, 15).

Rona and associates (12), observed that myocardial lesions of marked severity could be produced in rats by using IPT doses of 85 *mg/kg* once daily for two days However, this dose regime in our earlier studies (7) failed to reveal consistently ECG evidence of such severe lesions. Recently, higher doses to the extent of 160 *mg/kg* and 200 *mg/kg* subcutaneously or intraperitoneally have been used by Sahyoun and Hicks (14) and Davidson (4) respectively to induce severe myocardial lesion as judged by ECG parameters. But these high doses are known to increase mortality rates (12) and lesions of only severe degree were obtained. Less attention seems to have been paid to produce the lesions in a graded fashion.

A method producing better gradability of the lesions, if available, would ensure more quantitative intergroup comparisons and thereby permit better evaluation of even such drugs whose potential activity is relatively weaker and are, therefore, likely to be missed if assessed by such method as would produce lesions of only severe degree. In view of this, we have studied the effects of frequency of a low and high dose of IPT on the lesion severity.

In the present work, therefore, systematic study was undertaken to determine the number of doses of IPT and the duration for which it should be given to induce graded lesions of myocardium in rats.

## MATERIALS AND METHODS

Male albino rats weighing between 200 to 250 *g* were used in this study. IPT sulphate dissolved in the distilled water was administered subcutaneously once daily for one to four consecutive days in low dose (85 *mg/kg*), or for one to two days in high dose (170 *mg/kg* body weight).

Twentyfour animals of group A receiving IPT in the dose of 85 *mg/kg* were equally divided into four groups ($A_1$, $A_2$, $A_3$, $A_4$). Single dose of the drug was administered to the rats in group $A_1$ whereas two, three and four consecutive doses were given at 24 hr. intervals to rats in group $A_2$, $A_3$ and $A_4$ respectivetly.

Twelve animals of group B were exposed to the high dose (170 *mg/kg*) of IPT, half of which ($B_1$) received a single dose, while the other half ($B_2$) were administered two doses at an interval of 24 hr.

The control rats, six in a group corresponding to each experimental group referred above, received same volume of distilled water in a single or more doses at identical intervals.

All animals were sacrificed 24 hr after the last dose of IPT (experimental group) or distilled water (control group.) Following investigations were carried out.

*Electrocardiogram (ECG) recording :* Under light ether anaesthesia ECGs were recorded from each animal using limb leads I, II, III, aVR, aVL, aVF and precordial leads as reported in previous study (7).

*Serum creatine kinase (Serum CPK) :* Before the animal was sacrificed, blood was collected from the left ventricle of the animals. Serum CPK (Code No. EC 2.7.3.2) was estimated by the method of Okinaka *et al.* (10).

*Gross and microscopic examination :* After gross examination, the hearts were fixed in Bouin's fluid for histological processing. Gross and microscopic lesions were classified according to the gradations suggested by Rona *et al.* (12) from which the mean grading in each group was calculated. Krushal Wallis test (nonparametric test) was used for statistical analysis.

Correlation between Serum CPK level and severity of necrosis was determined by correlation coefficient.

## RESULTS

*ECG recordings :* ECG monitoring during the IPT administration revealed clear indication of myocardial ischaemia. In the group A, increase in QRS and QT intervals was observed at the end of 24 hr. Presence of small Q wave in lead I, aVR and aVL was consistently noticed in ECGs recrorded at 48 hr. Q wave deepened progressively at the end of 72 and 96 hr and was noticed in precordial leads also. The amplitudes of R wave also gradually increased, reaching a peak of $1.075 \pm 0.05$ mV after 96 hr. in $V_1$ and $V_2$. The amplitude of T wave also showed significant increase after 72 and 96 hr.

In animals of group $B_1$, recordings from lead I, aVR, aVL, $V_3$ and $V_4$ showed presence of Q wave. In group $B_2$, the Q wave further deepened and in addition, a prominent R wave (lead $V_1$, $V_2$) and upright T wave (lead I, aVL, $V_3$, $V_4$ & $V_5$) were observed in all animals (Fig. 1).

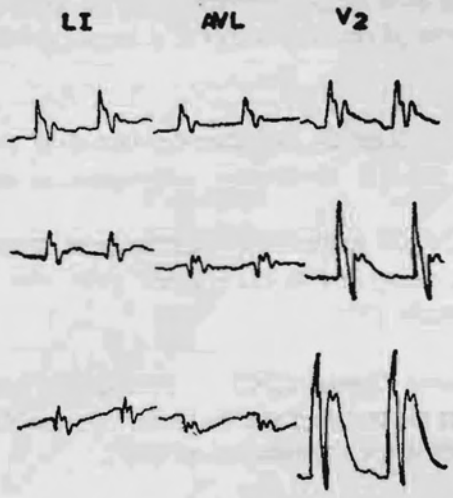


Fig. 1 : Representative ECG records of group A rats. Gradual increase in amplitudes of Q wave in leads I and aVL and of R and T waves in $V_2$ seen 24 hr (upper tracing), 48 hr (middle tracing) and 72 hr (lower tracing) after 85 *mg/kg* of IPT sulphate.

The control animals did not show any ECG evidence of myocardial ischaemia.

*Myocardial lesions :* IPT produced a dose-dependent necrosis of the myocardium. With low dose (group A), the lesions were noticed even after one day of IPT treatment and extension of IPT treatment by each day, caused a significant increase in the degree of lesion as compared to that on the previous day. Using high dose (group B), dose dependent but higher grades of lesions could be obtained earlier (Table II).

TABLE I : Heart rate, P–R interval, QRS complex and Q–T interval in control and experimental rats. Values are mean ± SEM.

| *Group* | *Heart rate beats/min* | *P–R intervals in msec* | *QRS complex in m sec* | *Q–T interval in msec* |
|---|---|---|---|---|
| Control | 419±16.5 | 41±1.50 | 21±1.7 | 70±6.8 |
| $A_1$ | 421±8.9 | 41±0.08 | 22±4.6 | 72±3.8 |
| $A_2$ | 424±11.6 | 41±2.60 | 24±1.3 | 73±3.9 |
| $A_3$ | 430±17.7 | 42±3.00 | 26±17*** | 76±4.7** |
| $A_4$ | 439±26.9 | 41±1.50 | 26±1.8*** | 77±1.0*** |
| $B_1$ | 427± 9.1 | 41±2.00 | 24±4.0 | 74±2.2* |
| $B_2$ | 440±15.0 | 42±1.20 | 26±1.6** | 77±2.1*** |

*P<0.05; **P<0.01; ***P<0.001.

TABLE II : Effects of repeated doses of isoproterenol on the extent of myocardial lesion and serum CPK levels in the albino rats.

| | *A. 85 mg/kg IPT for* | | | | *B. 170 mg/kg IPT for* | | *P value (Intergroup comparison)* |
|---|---|---|---|---|---|---|---|
| | *1 day ($A_1$)* | *2 days ($A_2$)* | *3 days ($A_3$)* | *4 days ($A_4$)* | *1 day ($B_1$)* | *2 days ($B_2$)* | |
| *Myocardial lesion* | | | | | | | |
| Grade | G M | G M | G M | G M | G M | G M | |
| 0 | — — | — — | — — | — — | — — | — — | |
| 1 | 6 6 | — — | — — | — — | — — | — — | |
| 2 | — — | 5 5 | — — | — — | 2 2 | — — | |
| 3 | — — | 1 1 | 4 4 | — — | 4 4 | — — | |
| 4 | — — | — — | 2 2 | 6 6 | — — | 6 6 | |
| Mean grade of the group | 1.0±* 0.0 | 2.2* 0.2 | 3.3±* 0.2 | 4.0±* 0.0 | 2.7±* 0.2 | 4.0±* 0.0 | $A_1$ : $A_2$<0.01<br>$A_2$ : $A_3$<0.01<br>$A_3$ : $A_4$<0.02<br>$B_1$ : $B_2$<0.01 |
| Serum CPK (I.U. per litre) | 60.0±** 1.2 | 96.0±* 1.7 | 140.0±* 1.0 | 198.0±* 1.3 | 81.3±* 0.8 | 171.0±* 1.5 | $A_1$ : $A_2$<0.01<br>$A_2$ : $A_3$<0.001<br>$A_3$ : $A_4$<0.001<br>$B_1$ : $B_2$<0.001 |

*P<0.001; **P<0.01 in relation to the control values (myocardial lesion grade = 0), (CPK = 55.0 ± 0.7 I.U. per litre serum). Data represents mean ± S.E. values.
No. of experiments : Six in each group; G = Gross; M = Microscopic.

*Serum CPK levels :* Serum CPK levels showed a rise over the normal levels after IPT administration. The rise was directly related to the dose as well as to the number of doses, each additional dose causing a highly significant increase over the previous day's levels (Table I). Our results also demonstrate highly positive co-relation ($r=0.9249$; $P<0.01$) between rise of serum CPK and severity of necrosis (Fig. 5).

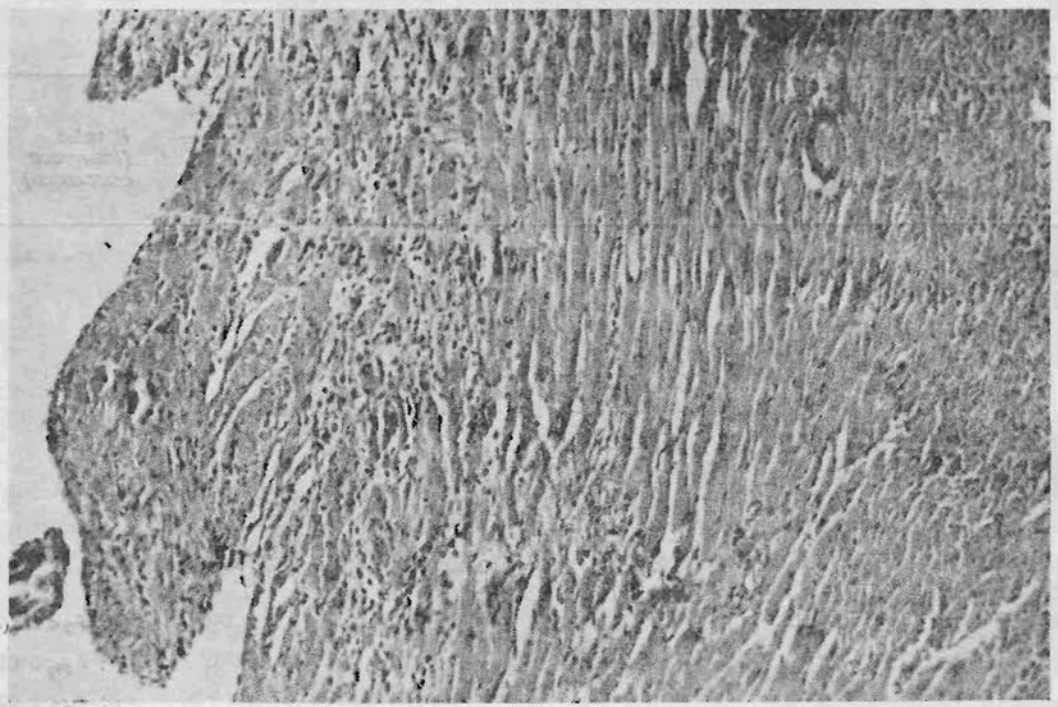

Fig. 2 : Heart muscle showing evidence of myocarditis with diffuse infiltration by lymphocytes and polymorphs after 48 hr of 85 *mg/kg* of IPT for two consecutive days (H & E x 150).

*Viscera :* In all animals marked congestion with petechiae was observed in abdominal viscera. Pulmonary edema and haemorrhage was also noted in 40 per cent of rats receiving the dose of 170 *mg/kg* but these findings were not observed in animals treated with 85 *mg/kg*.

*Mortality :* Combined mortality rate in group A is 12.4%, with $A_4$ exhibiting 8.3% and $A_3$, 4.1%. The mortality rate in group $B_1$, remained same as seen in $A_4$ (8.4%), but the rate in group $B_2$, increased to a highly significant value of 41.6%.

## DISCUSSION

The results of this study show that two consecutive low doses of IPT sulphate fail to produce cardiac lesion of marked severity in rats. On the other hand, administration

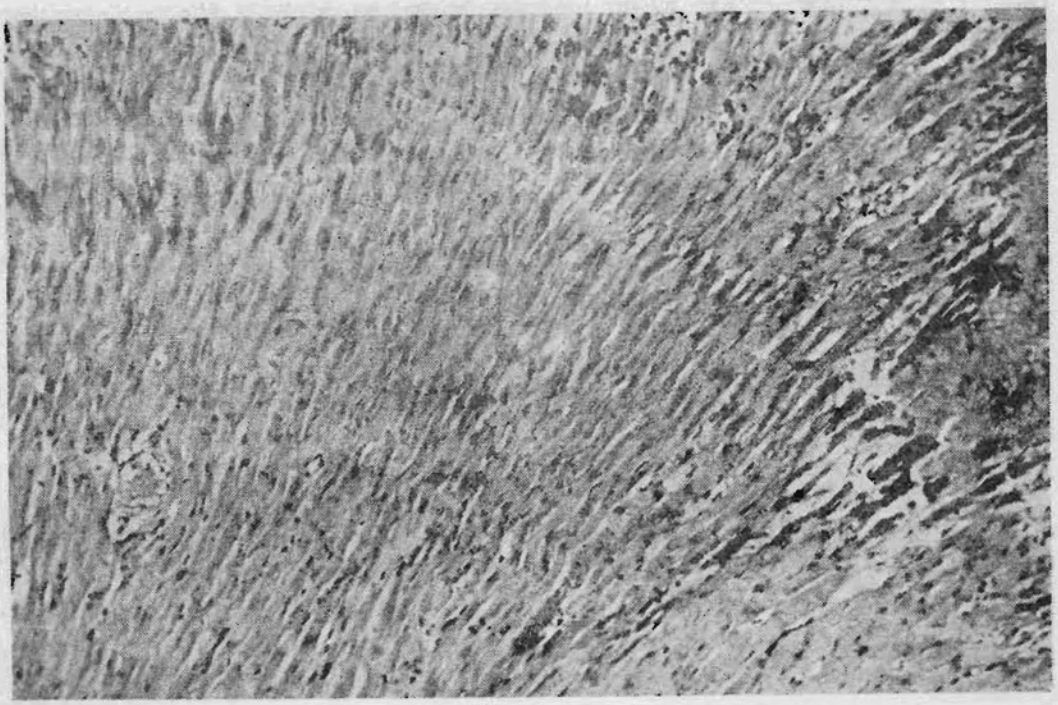

Fig. 3 : Heart muscle showing an area of necrosis of myocardial fibres at upper right side after 72 hr of 85 *mg/kg* of IPT for three consecutive days ( H & E x 150).

of four consecutive doses leads to produce mild to severe lesion in every rat. Our results differ from those of Rona *et al.* wherein they demonstrated only severe lesions after two doses of IPT hydrochloride in the dose of 85 *mg/kg* of body weight Systematic study of the lesion at regular intervals after administration of repeated consecutive doses has revealed the dose dependent effect of this drug on severity of myocardial lesion. Using 25 *mg/kg* IPT per day in Syrian Hamsters, Handforth and Halifax (5) showed similar dose-dependent effects leading to massive necrosis. They have demonstrated that severe local ischaemia precedes the development of necrosis after IPT and subsequent lesion produced is in fact an infarct and not an "infarct like necrosis" as indicated by Rona *et al.* (12).

Requirement of multiple low doses of IPT sulphate (85 *mg/kg*) for production of mild to severe lesion in our study (group A) in contrast to that of Rona *et al.* (12) where they produced only severe lesions may be due to strain and/or environmental (1, 2, 17) variation, and/or the difference between the bioavailability of the salts of IPT used by

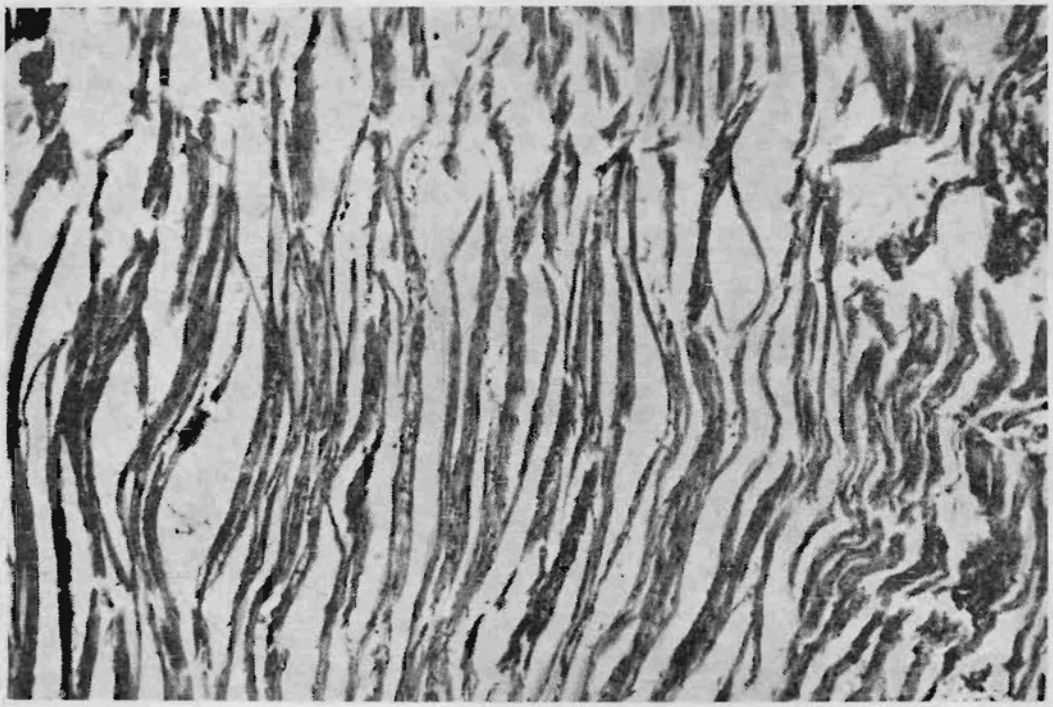

Fig. 4 : Fragmentation of myocardial muscle fibers after 96 hrs of 85 *mg/kg* of IPT for four consecutive days. (H & E x 150).

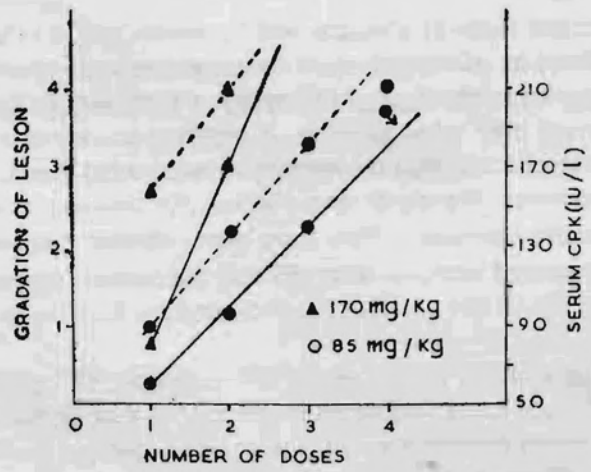


Fig. 5 : Effects of 85 *mg/kg* (0) and 170 *mg/kg* (▲) IPT sulphate on serum CPK levels (—) and grades of myocardial lesion (— — — —).

Rona *et al.* (hydrochloride) and in present studies (sulphate). There is a wide difference in the molecular weight of these two salts of IPT. Development of hypoxic tolerance to IPT (7) when given repeatedly dose not appear to be involved at leasst upto 4 doses as is evident from the dose–dependent increase in the severity of myocardial lesion in the present studies.

Administration of high doses of IPT sulphate (170 *mg/kg* body weight) to B group of rats led to severe lesion (grade II, III and IV). Severity of lesion is demonstrated by ECG findings and elevated serum CPK levels in experimental group as these parameters are used in clinical cases (6). ECG findings of similar nature were demonstrated by other workers (7, 8) on using high dose (170 *mg/kg* body weight) of IPT for two consecutive days.

Tajuddin *et al.* (17) have also shown six fold increase in serum CPK level on IPT induced myocardial necrosis. Moreover, Normis *et al.* (9) have demonstrated a good positive correlation of serum CPK with clinical indices of the extent of myocardial damage. Our findings on animal experimentation are in agreement with the above observation.

Rona *et al.* (5) have produced severe lesion, by using IPT hydrochloride (85 *mg/kg* body weight), for two consecutive days. But in our study, production of graded lesions, varying from mild to severe degree is achieved with IPT sulphate (85 *mg/kg* body weight) administered for four consecutive days. However, this work also demonstrates that for inducing exclusively severe lesion in shorter period, high dose of IPT (170 *mg/kg* body weight) can be used. But such a procedure leads tc high mortatlity rate. Such high mortality may be due to the development of severe cardiac lesion cf sudden onset and possibly involvement of other vital organs as seen in our studies. Moreover, the lesions are not produced in a graded fashion (only severe lesions were produced by this method).

Based on the differing results of the two experimental groups, administration of low dose (85 *mg/kg* body weight) of IPT sulphate for four consecutive days seems to be a safe and reliable procedure for inducing lesion in a graded fashion. In view of various factors capable cf modifying the development of IPT induced myocardial necrosis (13), a method involving four doses as described above may provide greater flexibility and prove more readily adaptable to environments of individual laboratories than a two dose method of producing myocardial lesions.

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