The Effect of Propranolol on Canine Myocardial CPK Distribution Space and Rate of Disappearance

JOHN A. CAIRNS, M.D., F.R.C.P.(C), AND GERALD A. KLASSEN, M.D., F R.C.P., (C)

SUMMARY Canine myocardial CPK was partially purified and injected into 11 conscious mongrel dogs. From serial serum CPK measurements in each dog, mean Kd was calculated as 0.0047 ± 0.0009 (±SD) min⁻¹. Correlation coefficients indicated that CPK disappearance rate was well described by a single exponential expression. Kd measured on consecutive days in four dogs varied minimally. CPK distribution space ranged from 74 to 134% of plasma volume. Propranolol loading with 0.3 mg/kg or 2 mg/kg, followed by hourly maintenance doses, resulted in increased Kd in eight of ten dogs, mean Kd rising from .0048 min⁻¹ to .0059 min⁻¹ (P < 0.02). Propranolol appeared to increase plasma volume but had no significant effect on the relationship of CPK distribution space to plasma volume. If the serial CPK technique were used to measure infarct size, using an average Kd, propranolol might produce artifactual reduction of infarct size measurement by increasing Kd and possibly by increasing plasma volume. The obligation to assess the effect upon CPK Kd and distribution space of an agent designed to limit infarct size is apparent.

SOBEL AND SHELL have described a technique for estimating the depletion of myocardial creatine phosphokinase (CPK) occurring in the course of acute myocardial infarction.¹-³ The technique is based on the kinetics of the CPK released by the damaged tissue. The estimate of myocardial CPK depletion is dependent on the use of an appropriate parameter characterizing CPK disappearance from the vascular compartment and verification that a valid approximation of the parameter can be obtained in order to calculate aggregate release of CPK from serial serum CPK values. The CPK disappearance from the vascular compart-

References

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Methods

Studies were carried out on 11 conscious mongrel dogs of mean weight 16 kg (range 13.6 – 19.1 kg). A small sedating dose of intravenous diazepam (mean 0.2 mg/kg) was administered. A sampling cannula was maintained in the internal jugular vein using a heparin trap. Heparin has been reported previously to reduce CPK activity. In a series of experiments we have been unable to demonstrate any difference between heparinized and nonheparinized serum samples. In any case, the amount of heparin in the samples was insignificant because of flushing of the trap prior to obtaining each sample. Canine myocardial CPK had been previously extracted from dog hearts by the method of Noda et al., yielding a lyophylate containing 115,000 IU CPK/g (44.2 IU/mg protein). This represented a 100-fold increase in CPK activity per gram. The yield of CPK activity was not determined. Electrophoresis on cellulose acetate with subsequent fluorescence scanning revealed that the extract contained 90% MM and 10% MB. Injection of the lyophylate into dogs led to recovery of 95% of injected CPK activity in controls. The lyophylate was kept in air tight containers at 4°C and when analyzed 18 months after preparation, the enzyme content had not changed significantly. For use, the lyophylate was dissolved in sterile distilled water and injected by peripheral vein as a bolus of 1000–2500 IU CPK/dog (mean 1725 IU/dog). Serial 1.5 ml serum samples for CPK analysis were obtained every 15 minutes for two hours, and hourly for 11 additional hours. Samples were allowed to clot at 4°C, were centrifuged and were either assayed that day or were frozen and assayed the subsequent day. CPK activity was measured by the Rosalki method using Eskalab bulk reagents at 37°C or Calbiochem Statpacks at 30°C.

The mathematical analysis of the serial serum CPK values was designed to approximate the previously established approaches of Sobel and co-workers. All CPK values were corrected by the subtraction of mean background CPK level before mathematical analysis (SK1 = 90, Calbiochem = 60). Baseline levels of CPK were determined in each dog and values were followed after they returned to the normal range in many. As has been observed in the work of others we found considerable variation in baseline serum CPK levels from one time to another in a given dog and between dogs prior to CPK injection. For this reason, all baseline CPK values were averaged and this mean value was taken as the upper normal limit and subtracted from all serum CPK values prior to regression analysis. Corrected serum CPK values were plotted against time on semi-log paper, and CPK disappearance rate (Kd) was determined using the statistics program of a Hewlett Packard desk computer to develop an expression for the monoexponential decay of CPK. A value for Kd was obtained in each case as a parameter of the degree to which the exponential regression was a good representation of the data. Generally, the sampling protocol was terminated before CPK returned to the normal range. In those experiments where serum sampling was continued until CPK values were oscillating in the normal range, a final value was chosen which was still above normal. Comparisons of Kd in a given dog were made on the basis of the most closely comparable final CPK values, below which no values were utilized. Distribution space was determined by dividing the known amount of injected CPK by the theoretical initial concentration obtained from the y-intercept of the exponential disappearance curve.

Plasma volume was measured using either T185 dye or 141I labelled albumin. The indicator was injected at the five hour point of the experiment and the blood sample for calculation of dilution was drawn ten minutes later.

On a subsequent day, each dog was given intravenous propranolol 0.3 mg/kg (six dogs) or 2 mg/kg (four dogs) and the CPK was again injected. The sampling, analytical, and plasma volume measurement protocols of the first day were repeated. Propranolol blood level was maintained by hourly injection of 20% of the loading dose.

In four dogs, the CPK injection protocol was carried out on two successive days before the administration of propranolol on the third day.

The efficacy of propranolol in acute myocardial infarction has been studied primarily from two aspects: the effect on prevention of ventricular fibrillation, and the effect on infarct size. We elected to look at the effects on Kd of propranolol in modest dosage (0.3 mg/kg) as used in ventricular fibrillation prevention studies and in large dose (2.0 mg/kg) as used in the infarct size modification studies. The larger dose is seven to 14 times that required to completely block the beta adrenergic tachycardia of maximal exercise in humans. The dosages given previously have been found to produce blood levels in dogs of about 180 ng/ml (0.3 mg/kg) and 1,200 ng/ml (2 mg/kg). Blood levels of propranolol were not monitored in the current study.

Results

Control Data

Mean control CPK disappearance rate (Kd) for the 11 dogs was 0.0047 ± 0.0009 (mean ± sd) min⁻¹ (table 1). The Kd values approach 1.5 in each case indicating that CPK disappearance is well described by a monoexponential expression. The possibility was considered that a biexponential expression might be a better description of the data, if disappearance were occurring from a two compartment system. A computer program was used by which an exhaustion technique successively peels off more and more

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Kd (min⁻¹)</th>
<th>r²</th>
<th>Day 1</th>
<th>Day 2</th>
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<tbody>
<tr>
<td>1</td>
<td>0.0044</td>
<td>0.97</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>0.0054</td>
<td>0.99</td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td>0.0048</td>
<td>0.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.0038</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.0040</td>
<td>0.97</td>
<td>0.0040</td>
<td>0.99</td>
</tr>
<tr>
<td>6</td>
<td>0.0047</td>
<td>0.96</td>
<td>0.0054</td>
<td>0.99</td>
</tr>
<tr>
<td>7</td>
<td>0.0061</td>
<td>0.99</td>
<td>0.0061</td>
<td>0.97</td>
</tr>
<tr>
<td>8</td>
<td>0.0038</td>
<td>0.99</td>
<td>0.0044</td>
<td>0.90</td>
</tr>
<tr>
<td>9</td>
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<td>0.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.0045</td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0.0060</td>
<td>0.98</td>
<td></td>
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</table>

Mean ± SD Kd = exponential disappearance rate; r² = correlation coefficient.
points from the curve attempting to develop a biexponential expression which gives rise to a higher \( r^2 \) than does the monoexponential expression, indicating a better description of the data. Of the 25 comparisons, the biexponential was superior in seven, but in only one of these seven was the improvement in the fit of the data significant. The CPK disappearance may thus be expressed as a monoexponential decay with insignificant improvement in the expression using the more complex biexponential model.

Although there was considerable inter dog variation of \( K_d \), the variation in a given dog from day to day was minimal (fig. 1, table 1).

There was relatively close agreement between the measured plasma volume (by \( T_{18} \text{Na} \) dye or \( I^{131} \) labelled albumin) and the calculated CPK distribution space (from the y-intercept of the exponential disappearance curve) (table 2). The calculated distribution space ranged from 74–134% of the measured plasma volume, but there was no statistically significant difference between the two volumes. It is therefore apparent that the CPK distribution space is very close to that of plasma.

Propranolol Data

Propranolol was given to 10 of the 11 dogs. Mean \( K_d \) rose from 0.0048 to 0.0059, a statistically significant increase for the group (\( P < 0.02 \), paired t-test) (figs. 1 and 2). The \( K_d \) did not change in one dog and fell 26% in another. There were no differences apparent between the effects of the two doses of propranolol (0.3 mg/kg or 2 mg/kg).

The measured plasma volume (\( T_{18} \text{Na} \) dye or \( I^{131} \) labelled albumin) was again compared to calculated CPK distribution space (from the y-intercept of the exponential disappearance curve) on the day of propranolol administration.
The distribution space of CPK was initially determined by Sobel and Shell to be a volume equal to 11.4% of body weight, or roughly three times plasma volume.\(^9\) However, their CPK disappearance curves were biexponential with a rapid early Kd (thought to represent diffusional disappearance from a plasma into the full distribution space) and a subsequent slower sustained Kd (thought to represent metabolic disappearance from the distribution space). We found that a single exponential closely approximated all CPK values from 15 minutes of injection and that the y-intercept indicated distribution in a space approximately equal to plasma volume. It is possible that the early findings of Sobel and Shell were the result of some denaturation of their injected CPK. In fact, recent work by the Sobel group now reports a CPK distribution space equal to plasma volume.\(^9\)

Sobel and Roberts have shown that reduction of the cardiac output to 40% of normal, or ligation of the hepatic or renal artery, does not alter Kd.\(^13\),\(^14\) However, they did find that administration of the reticuloendothelial system blocker zymosan produced marked reduction of Kd.\(^14\) They have concluded that CPK metabolism occurs in the reticuloendothelial system. Our finding that propranolol increases Kd suggests that propranolol may have some influence on the reticuloendothelial system or on the access of CPK to the reticuloendothelial system perhaps by redistribution of cardiac output. The significance of our finding is that use of a control or assumed Kd in the calculation of the amount of myocardial enzyme depletion in a propranolol treated subject may lead to underestimation of the amount of enzyme depletion and an artificial improvement of efficacy of the drug. If two dogs, A and B (fig. 3) undergo myocardial damage of exactly the same evolutionary pattern and size, but with dog B receiving propranolol, the curves of serial

### Table 3. Exponential Disappearance Rate of CPK: Effect of Propranolol

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Control</th>
<th>Propranolol 0.3 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kd (min(^{-1}))</td>
<td>r(^2)</td>
</tr>
<tr>
<td>1</td>
<td>.0044</td>
<td>.97</td>
</tr>
<tr>
<td>2</td>
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<td>.99</td>
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<tr>
<td>3</td>
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<td>.91</td>
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<tr>
<td>4</td>
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<tr>
<td>10</td>
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<td>.97</td>
</tr>
<tr>
<td>11</td>
<td>.0060</td>
<td>.98</td>
</tr>
<tr>
<td></td>
<td>Propranolol 2.0 mg/kg</td>
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</tr>
<tr>
<td>5</td>
<td>.0040</td>
<td>.97</td>
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<tr>
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<td>.0051</td>
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<tr>
<td>SD</td>
<td>.0008</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>.0002</td>
<td></td>
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</tbody>
</table>

**Abbreviations:** Kd = exponential disappearance rate; r\(^2\) = correlation coefficient.

(table 2). Again, there was a reasonably close agreement between the two volumes, calculated CPK distribution space ranging from 72–212% of measured plasma volume. The volumes are not statistically different, indicating that propranolol administration did not alter the approximate equivalence of distribution space and plasma volume.

Measured plasma volume increased significantly from control day to propranolol administration day (P < 0.05, paired t-test) (table 2), indicating that although the approximate equivalence of plasma volume and CPK distribution space was not altered by propranolol, the actual volume of distribution space may have been increased. The experiments were designed to study the effect of propranolol on the relationship of CPK distribution space to plasma volume and therefore on the day of propranolol administration plasma volume was measured only once, five hours after starting the drug, while the control plasma volume measurement was that obtained the previous day. The increment of plasma volume may have been the result of propranolol, but without a control plasma volume measurement immediately before propranolol administration this conclusion is tentative.

### Discussion

The initial experiments of Sobel and Shell demonstrated that CPK disappearance in the dog could be approximated by a monoeponential expression and that the disappearance rate (Kd) estimated from such approximations permitted estimation of myocardial enzyme depletion from serial serum CPK values.\(^8\) Our observation of a mean Kd of 0.0047 ± 0.0009 min\(^{-1}\) agrees closely with their determination of 0.0045 ± 0.0010 min\(^{-1}\).\(^8\) We have observed a similar close approximation of CPK disappearance by a monoeponential regression, without significant improvement in description of the data by a biexponential regression. The evidence that Kd does not vary significantly from day to day in a given dog supports the assumption of a constant Kd throughout the 48 hours or longer sampling sequence required to determine the amount of enzyme depletion and confirms identical sequential studies of Sobel and Roberts.\(^8\)

![Figure 3](http://circ.ahajournals.org/content/37/4/287/suppl/DC1/1)
CPK levels will be different because of the larger Kd in dog B. If the actual Kd in each dog is calculated from the exponential downslope of the serial CPK curve and used to calculate the amount of myocardial enzyme depletion, the depletion will be seen to be identical in amount and pattern of evolution. However, if the control Kd is used to calculate the amount of enzyme depletion in the propranolol treated dog, the amount will be underestimated and it will appear that propranolol has reduced enzyme depletion. To the extent that enzyme depletion may be related to quantitative myocardial necrosis and further to infarct size, the intervention would be wrongly judged to be efficacious. There would be no problem were the actual Kd to be measured and utilized in each case. However, if Kd is assumed the problems are apparent.

The distribution of CPK in plasma volume requires that the effect upon plasma volume of an intervention designed to limit infarct size must be considered when serial CPKs are being used to assess modification of myocardial damage. Expansion of the plasma volume will lead to underestimation of myocardial damage; contraction of the plasma volume will lead to overestimation of myocardial damage. It may be that propranolol expands the plasma volume, although the present study protocol was not designed to examine this effect specifically.

Delineation of the nature of CPK disappearance from the circulation is necessary to obtain a parameter for use in estimating amount of enzyme depletion by analysis of serial serum CPK changes. The monoexponential disappearance of CPK (originally observed by Sobel and Shell and confirmed in the present study) greatly facilitates such estimations. The values for Kd in the dog determined in this study agree closely with those previously reported by Sobel and Shell. Day to day variation of Kd in a given dog would give rise to errors in myocardial damage calculation when sampling may extend over 48 hours. The minimal variation demonstrated indicates that such errors would be small. The effect of an intervention designed to limit infarct size on Kd or distribution space of CPK must be investigated before any conclusions can be drawn about its influence on myocardial damage. These effects must be incorporated into the calculation of amount of enzyme depletion for conclusions to be meaningful. It appears that propranolol, with its effect on Kd and possibly upon distribution space of CPK, has the potential for the artifactual reduction of amount of enzyme depletion and myocardial damage, if these effects are not taken into account.

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