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Electrocardiographic changes induced by levamisole hydrochloride in the guinea-pig

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Summary

The effects of various i.v. doses of levamisole on the ECG of the anaesthetized guinea-pigs were determined. At 6 mg/kg there were no changes in the heart rate, P–R and Q–T intervals and no cardiotoxic effects were observed. At 10 and 20 mg/kg these parameters were affected little but cardiotoxic effects, mainly ventricular extrasystoles lasting from a few seconds to several minutes, were observed in five out of 10 animals that received either of the two doses. No animal died during the 1 h period of observation. It would appear that levamisole is much less toxic to the guinea-pig heart than to the heart of the rat.

Résumé

On a déterminé les effets de différentes doses de levamisole, administrées par voie i.v., sur l'électro-cardiogramme d'un cobaye anésthésié. Une dose de 6 mg/kg ne change ni le rythme cardiaque, ni les intervalles P–R et Q–T et aucun effet cardiotoxique n'est observé. Des doses de 10 et 20 mg/kg affectent peu ces paramètres, mais des effets cardiotoxiques, principalement des extrasystoles ventriculaires d'une durée allant de quelques secondes à plusieurs minutes, ont été observés chez cinq des 10 animaux qui ont reçu l'une de ces deux doses. Aucun animal n'est mort au cours de l'heure d'observation. Il apparaitrait que levamisole est beaucoup moins toxique pour le coeur du cobaye que pour celui du rat.

Introduction

Levamisole hydrochloride, a potent anti-ascaris and anti-hookworm agent, was shown to possess anti-arrhythmic properties comparable with those of lignocaine [1]. However, further studies in this laboratory designed to determine the safety factor as regards its ability to revert to sinus rhythm, $BaCl_2$-induced ventricular dysrhythmia, showed that its safety margin and also its efficiency may be low. Thus, while 6 mg/kg was able to revert $BaCl_2$-induced ventricular dysrhythmia to sinus rhythm in three out of 10 rats, 10 mg/kg did not achieve greater success. Moreover, at 20 mg/kg it completely failed to revert to sinus rhythm $BaCl_2$-induced ventricular dysrhythmia in any of the 10 rats. Instead, all the 10 rats died from ventricular fibrillation. It was suspected that levamisole might be causing cardiac arrhythmias at such a high dose. A study of ECG changes induced in the rat by various doses of levamisole was thus undertaken. The result of that study [2] showed that levamisole could be cardiotoxic even at 2 mg/kg i.v. The rat, however, is rather notorious for the wide individual variation in the response of the heart to drugs. Guinea-pigs, however, show very little individual variation. Thus, the guinea-pig is a good animal model for studying effects of drugs on the heart. This study was therefore embarked upon in order to determine if there are also any significant species differences in the responses of the guinea-pig and rat heart to levamisole.

Materials and methods

Twenty guinea-pigs weighing between 300 and 375 g were used. The animals were anaesthetized with thiopentone sodium (30 mg/kg) administered intraperitoneally. The guinea-pigs were then placed in a supine position with all four limbs tied to a dissecting board. A longitudinal midline incision about 1 cm in length

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was made in the neck and the skin reflected laterally to expose one of the internal jugular veins. The vein was cannulated with a polythene cannula filled with heparinized saline (10 IU of heparin/ml of normal saline) and secured in place for drug administration.

The animal was then connected to an ECG machine (Narco-Biosystem Physiograph MK-III) by means of electrodes inserted subcutaneously into the right fore limb and left hind limb. ECG records were obtained from the lead II channel of the machine. The ECG took about 15–20 min to stabilize before levamisole was injected i.v. ECG records were obtained continuously for 1 min following the administration of levamisole, or normal saline in the case of controls, and thereafter 5-sec records were obtained every minute during the next 4 min and every 5 min during the next 55 min. Paper speed was set at 5 cm/sec.

The animals were divided into four groups of five animals each. Each animal in any group received the same dose of levamisole, 6, 10 or 20 mg/kg, and the control group received normal saline (1 ml/kg) which was approximately the volume of fluid given to the drug-treated animals.

Details of ECG changes were evaluated according to standard practice and the parameters measured included heart rate; P–R, Q–T and R–R intervals; amplitude and duration of the P wave.

As the Q–T interval varies with heart rate, a corrected Q–T interval (QT_c) was obtained using the relationship:

$$QT = K \surd \text{R–R interval.}$$

According to Onuaguluchi *et al.* [3]

$$QT_c \text{ corresponding to a } QT_1 \text{ interval} = \frac{QT_0 \times \surd R_1\text{–}R_1}{\surd R_0\text{–}R_0}$$

where QT_0 = observed Q–T interval at equilibration;
QT_1 = observed Q–T interval after equilibration;
R_0–R_0 = R–R interval in msec at equilibration;
R_1–R_1 = R–R interval in msec after equilibration.

The real difference in Q–T interval from value at equilibration

$$= QT_0 - QT_c.$$

Therefore: percentage change

$$= \frac{QT_0\text{–}QT_c}{QT_0} \times 100.$$

The average percentage change ± s.e.m. for each time of assessment was calculated for all the animals which received each dose of levamisole and for the control group. It was thus possible to determine what the highest average percentage change was and when it occurred. The time course of effects was also plotted. The average percentage change ± s.e.m. over the whole 1-h period of observation was also calculated for each dose of the drug and for the control group.

Averages were expressed as means ± s.e.m. Student's unpaired *t*-tests were performed to compare results obtained from each dose of drug with the results from control experiments. A *P*-value of <0.05 was taken as indicating a statistically significant difference.

Results

Heart rate

In the control group there was an average percentage change in the heart rate of 0.54 ± 4.61. The range was between −15.72 ± 7.10 and 6.2 ± 3.81. At 6 mg/kg, levamisole induced an average percentage change of −2.88 ± 2.91. The range was between −17.58 ± 2.54 and 7.75 ± 4.50. At 10 mg/kg, the average was −4.36 ± 4.45 with a range of between −25.88 ± 6.59 and 3.03 ± 1.97. At 20 mg/kg, there was an average percentage change in rate of −9.90 ± 3.67 with a range of between −30.72 ± 2.84 and −2.14 ± 4.6. The differences between the controls and drug-treated animals were not statistically significant.

Atrial and atrioventricular conduction

In all the animals studied a P wave preceded the QRS complex at equilibration. The duration of the P wave could not be measured accurately because 5 cm/sec was the maximum paper speed obtainable. In three out of five in the control group, the P–R interval remained virtu-

ally unchanged at 40 msec for most of the period of observation. The average percentage change for the control was -4.97 ± 0.67. The range was between -1.66 ± 9.27 and -8.33 ± 8.32. In the drug-treated animals the P–R interval remained virtually unchanged throughout most of the 1-h period of observation in the group that received 6 mg/kg. The average percentage change was -2.57 ± 1.21 and the range was between -12.25 ± 6.25 and 6.25 ± 6.25. At 10 mg/kg, P–R interval remained unchanged in three of the five animals treated. In one of the remaining two animals the P–R interval was reduced from 80 msec to 60 msec within 1 min of injecting the drug and remained at this level for the rest of the period of observation. In the other animal, the P–R interval was reduced from 100 msec to 60 msec within 10 sec of administering the drug. It was further reduced to 40 msec 10 sec later and ventricular dysrhythmia supervened. The average percentage change at this dose level was -10.88 ± 0.48 with a range of between -8 ± 8.8 and -13.00 ± 8.31. At 20 mg/kg the P–R interval was also virtually unchanged during the 1-h period of observation in three of the five animals treated. In one of the remaining two animals there was a 25% reduction for most of the period and in the other animal there was an increase of 33%. The average percentage change was 2.84 ± 0.78 and the range was between 1.66 ± 9.27 and 13.31 ± 8.16.

Ventricular depolarization and conduction

In the five control animals the amplitude of the R wave and the width of the QRS complex remained virtually unchanged from the values at equilibration. At 6 mg/kg the amplitude of the R wave fluctuated by not more than 1 mm throughout the period of observation. There was widening of the QRS complex in one of the animals and ventricular extrasystoles supervened. At 10 mg/kg there was more marked fluctuation in the amplitude of the R wave. It was between 2 and 4 mm except in one animal in which the amplitude was virtually unchanged throughout the period of observation. There was no widening of the QRS complexes unless they belonged to ventricular extrasystoles. At 20 mg/kg again there were marked fluctuations in the amplitude of the R wave. There was occasional widening of the QRS complex in one of the five guinea-pigs in this group.

Ventricular repolarization

There was little difference between the average percentage changes in Q–T interval corrected for rate in the controls and the drug-treated animals. The average percentage increases were 0.56 ± 1.96 for controls and 2.22 ± 1.78, 2.66 ± 2.21 and 4.61 ± 1.40 for 6, 10, 20 mg/kg respectively. However, it was noted that the greatest change in the Q–T interval occurred during the first 60 sec following drug or normal saline administration. When we consider these changes, significant differences appear between the controls and drug-treated animals. Thus in the control group, the highest average percentage change was 5.56 ± 2.65. In the drug-treated group, the highest average changes were 13.99 ± 2.39 ($P < 0.05$); 15.03 ± 3.78 ($P < 0.05$) 21.12 ± 2.72 ($P < 0.01$) respectively for levamisole 6, 10 and 20 mg/kg. Figure 1 is the time course of effects on Q–T interval corrected for rate in the control group and in each group treated with levamisole 6, 10 or 20 mg/kg. There were no T wave changes in the controls or in the group treated with levamisole 6 mg/kg. At 10 and 20 mg/kg, transient increases in the amplitude and duration of the T wave occurred.

Cardiac dysrhythmic phenomena

No cardiac arrhythmia occurred in any of the control animals. One animal in the group that received levamisole 6 mg/kg had isolated ventricular extrasystoles at the thirteenth sec after injecting the drug. The other four guinea-pigs in this group did not have any cardiac arrhythmia.

At 10 mg/kg, ECG characteristics of cardiac dysrhythmia were noted in three of the five guinea-pigs in this group. One of them had bizarre QRS complexes which lasted for 5 sec and occurred about 2 sec after administering the drug. Another animal had isolated extrasystoles and missed ventricular beats (Mobitz type II heart block). The ECG became normal 1 min later and remained so until the end of the experiment. Figure 2 shows the effect of leva-

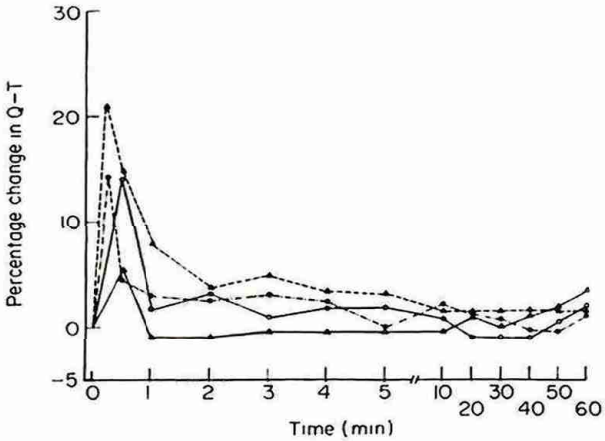

Fig. 1. Time course of effect of levamisole (△, control; ○, 6 mg/kg; ●, 10 mg/kg; ▲, 20 mg/kg) on Q–T interval in the guinea-pig. The changes are expressed as percentage difference between the Q–T interval at equilibration and the Q–T interval (corrected for rate changes – QT_c) at the time of observation.

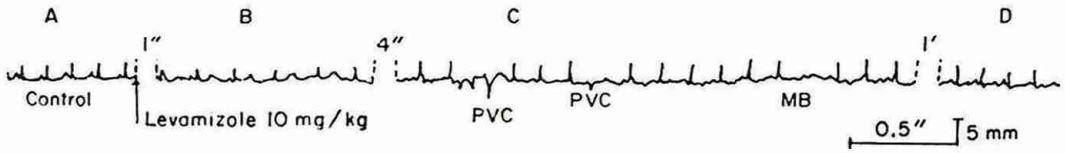

Fig. 2. ECG in a guinea-pig which received levamisole 10 mg/kg. Time course of effects: (C) shows the occurrence of premature ventricular contractions (PVC) and missed ventricular beats (MB) 5–8 sec after injecting levamisole. Sinus rhythm returned 1 min later and remained so until the end of the period of study.

misole 10 mg/kg in that guinea-pig. Figure 3 shows the type of cardiac dysrhythmia induced by levamisole 10 mg/kg in another guinea-pig. In that guinea-pig, runs of ventricular extrasystoles alternating with normal PQRS complexes (pulsus bigeminus) and lasting for 4 min became evident about 13 sec after administering levamisole (Fig. 3; sections C, D, E and F).

At 20 mg/kg, two out of the five animals in this group had ECG manifestations of cardiac dysrhythmia. One had ventricular extrasystoles lasting 2 min about 12 min after administering levamisole. The other animal had severe sinus bradycardia (Fig. 4; sections B and C), fol-

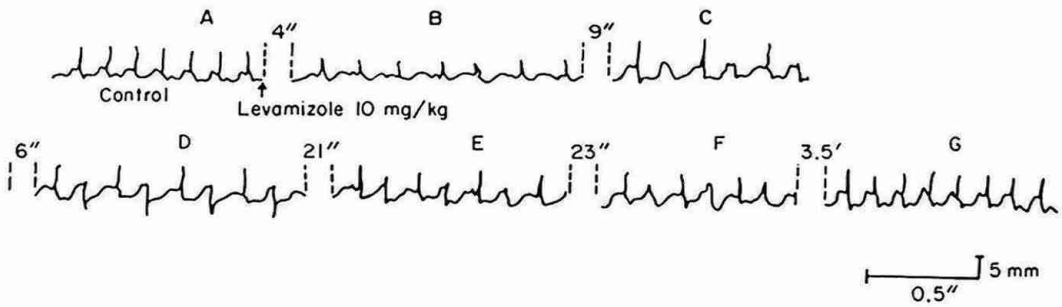

Fig. 3. Time course of effects of levamisole 10 mg/kg on the ECG of a guinea-pig. Pulsus bigeminus due to ventricular extrasystoles occurred about 13 sec after injecting levamisole and lasted for 4 min (C, D, E and F) before sinus rhythm was restored (G).

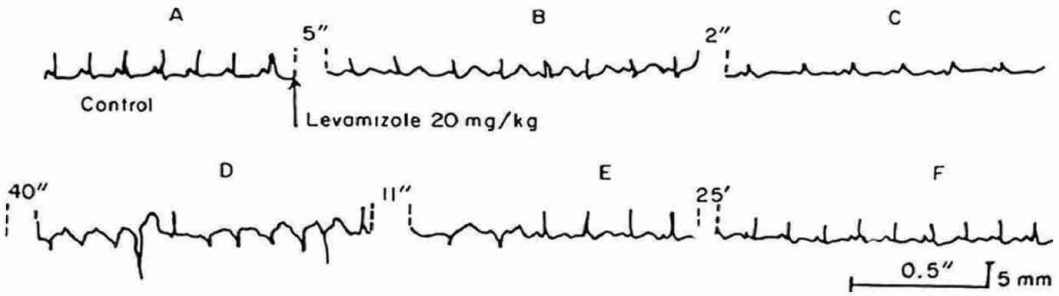

Fig. 4. ECG in a guinea-pig which received levamisole 20 mg/kg. (B) and (C) show severe sinus bradycardia followed 48 sec later by ventricular tachydysrhythmia (D and E). Sinus rhythm, however, returned after 25 min (F).

lowed 40 sec later by ventricular tachydysrhythmia (section D). This lasted about 25 min before sinus rhythm returned (section F).

Discussion

Levamisole caused a dose-dependent reduction in heart rate although this was not statistically significant even at the highest dose level studied. The duration of the P–R interval was affected little. As regards the Q–T interval, the average percentage change over the 1-h period of observation also did not differ from the controls. There were, however, statistically significant dose-dependent increases in the Q–T interval during the first 60 sec after drug administration. Widening of the QRS complexes was not generally a feature except as a feature of drug-induced ventricular extrasystoles. This is in contradistinction to what was noticed in the rat; widening of the QRS complexes often occurred and preceded the onset of ventricular tachydysrhythmia [2]. Ventricular extrasystoles appear to be the major cardiotoxic effect in the guinea-pig. The A–V bundle does not appear to be grossly affected unlike the rat where A–V blocks and dropped beats of Mobitz Types I and II frequently occurred, even at 2 mg/kg. In Mobitz Type I block the P–R interval progressively increases culminating in a dropped beat, but in Mobitz Type II block, the P–R interval is not progressively prolonged prior to the dropped beat [4].

The therapeutic dose of lignocaine in the treatment of ventricular arrhythmia in man is 2–3 mg/kg i.v. as a loading dose [5,6]. As the antiarrhythmic effect of levamisole is about equal to that of lignocaine or may even be slightly superior [1], the expected therapeutic antiarrhythmic dose of levamisole in man should therefore not be more than 3 mg/kg i.v. It is therefore of interest that levamisole (6 mg/kg i.v.) caused no cardiotoxic effect in the guinea-pig; the heart rate was only slightly lower than in the controls and there were no significant changes in the P–R or Q–T intervals.

None of the guinea-pigs which received levamisole died during the 1-h period of observation. However, in the rat [2], one out of four and two out of five of the rats that received 10 and 20 mg/kg, respectively, died during the 10–15 min period of observation. It would appear therefore that the guinea-pig would tolerate levamisole more than the rat. It is therefore important that ECG studies are carried out in higher animals such as the cat and the dog in order to determine whether the cardiotoxic effects of levamisole would be determined phylogenetically, with its toxic effects diminishing the higher the animal is on the phylogenetic ladder. If this were found to be so, it would encourage clinicians to embark on clinical trials designed to evaluate the antiarrhythmic effects of levamisole.

Acknowledgments

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