Electrocardiographic Changes Induced by Ivermectin in Guinea Pigs

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Summary

Guinea pigs were used to observe the possible side effects of ivermectin (500 µg/kg b.w.) on the heart by recording their electrocardiograms (ECG) at 24, 48 and 72 hours after its injection (experimental groups). Significant effects of ivermectin on the electrocardiographic parameters belong to limb lead II were compared to those of the control group. These were: the increases in amplitudes of P, S (only at 24 hours) and T waves, and also the lengthening (or "prolongation") in the durations of P and T waves, QRS complex (only at 24 and 48 hours) and PR and QTc intervals at 24, 48 and 72 hours. As a consequence of the prolonged durations mentioned above, statistically significant decreases of heart beats were found on the electrocardiograms recorded at 24 and 48 hours after ivermectin injection ($p<0.05$). In conclusion, the weak conductance in the heart of guinea pigs as an indicator of the prolongation in durations and intervals (negatively dromotropic) induced by ivermectin can be interpreted as its inhibitory effect on the sarcoplasmic reticulum Ca^{2+} -ATPase pump causing a late Ca^{2+} accumulation after systole due to the reduced amount of Ca^{2+}.

Introduction

Ivermectin has been used against many endoparasitic and ectoparasitic species in all domestic animals for almost two decades and in humans for the treatment of certain filarial tropical diseases (*Fisher & Helmut, 1992)*. The effects of ivermectin are based on the glutamate-gated CI^- channel that has only been found in certain invertebrates including nematodes (*Martin et al., 1997*). Although ivermectin is well tolerated in both human and animal, toxic effects may appear after using high doses (*Doody,* 1997). Some dog breeds, like the Collie, seem to be more susceptible to ivermectin toxicity. Common symptoms of ivermectin toxicity are vomiting, depression, mydriasis, ataxia, blindness, coma and sometimes death (*Edwards, 2003*). The use of ivermectin in the treatment of ectoparasitism in rabbits and guinea pigs has been studied by Mc Kellar *et al.* (*1992*).

Unfortunately few investigations about the side effects of ivermectin on the hearts of animals were conducted and they showed negative effects such as bradycardia in dogs (*Button et al., 1988)* and tachycardia in calves *Basudde, 1989)*. The purpose of this electrocardiographic study is to examine the possible side effects of ivermectin on the heart of guinea pigs secondary to its common use in domestic animals for the treatment of ectoparasitism.

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Materials & Methods

Ten male albino guinea pigs (Dunkin Hartley, Refik Saydam Hygiene Center, Ankara, Turkey) each weighing 300-350 g were used in this study. They were given food (Pellet, Purina, Istanbul,Turkey) and water *ad libitum.* Any anaesthetics were not applied during the processes. The limb leads were applied by means of a specially designed plate immobilising the animal in the supine position and

enabling good contact between three active electrodes and the neutral electrode with the limbs of the animal (*Cieślar et al., 1986*). A better contact between electrode surface and the skin of the limb was achieved by moistening the limb with gel (Berkim, Sonotact, Ultrasound Jell). Electrocardiographic recordings were made using Cardiofax ECG-6851 K (Nihon Kohden, Tokyo, Japan) at the speed of 50 mm/sec and sensitivity of 1 mV = 1 cm. Electrocardiograms of the animals were first recorded before any injection (control group). They then received a subcutaneously injection of ivermectin (Ivomek–Topkim Drug Co Ltd, Turkey) at the dose of 500 µg/kg b.w. and the electrocardiogram was recorded at 24, 48 and 72 hours after injection (experimental group).

Quantitative measurements such as; heart rates, amplitudes of P, R, S and T waves, duration of P and T waves, QRS complex and finally duration of PR and QTc (Corrected QT) (*Piotrovsky, 2005*) intervals were evaluated from limb lead II.

The control animals data were statistically compared with those from the experimental groups recorded at 24, 48 and 72 hours after injection of ivermectin and the experimental groups data were also compared together using repeated measures analysis of variance (*Mendenhall, 1971)*. Means were considered significantly different at $p< 0.05$. Data were expressed as means ± SEM.

Results

Measurements in limb lead II belong from the control animals and ivermectin (500 µg/kg b.w.) injected animals recorded at 24, 48 and 72 hours after injection are presented in Table 1.

Representative electrocardiograms from guinea pigs prior to drug injection (A), and 24 (B), 48 (C) and 72 hours (D) after injection are shown in Fig 1.

Table1. The electrocardiographic parameters of limb lead II taken before (control group) and after ivermectin (500 µg/kg b.w.) injection at 24, 48 and 72 hours (experimental groups) in the guinea pigs (n=10). Data were given as mean± SEM.

Parameters	Control	After drug administration		
		24 h	48 h	72 h
P wave duration (sec)	$0.025 \pm 0.002_a$	$0.043 \pm 0.005_b$	$0.048 \pm 0.004_b$	$0.040 \pm 0.003_b$
P wave amplitude (mV)	$0.150 \pm 0.011_a$	$0.210 \pm 0.007_b$	$0.205 \pm 0.017_b$	$0.195 \pm 0.016_b$
PR interval duration (sec)	$0.050 \pm 0.002_a$	$0.064 \pm 0.003_b$	$0.065 \pm 0.002_b$	$0.060 \pm 0.001_b$
QRS comp. duration (sec)	$0.018 \pm 0.001_a$	$0.027 \pm 0.003_b$	$0.028 \pm 0.003_b$	0.023 ± 0.002
R wave amplitude (mV)	0.560 ± 0.047	0.620 ± 0.042	0.670 ± 0.052	0.601 ± 0.066
S wave amplitude (mV)	$0.085 \pm 0.010_a$	$0.195 \pm 0.036_b$	0.155 ± 0.026	0.155 ± 0.024
T wave duration (sec)	$0.024 \pm 0.003_a$	$0.043 \pm 0.003_b$	$0.051 \pm 0.004_b$	$0.044 \pm 0.003_b$
T wave amplitude (mV)	$0.105 \pm 0.016_a$	$0.191 \pm 0.024_b$	$0.185 \pm 0.018_b$	$0.160 \pm 0.016_b$
QTc interval duration(sec)	$0.274 \pm 0.0028_a$	$0.313 \pm 0.0035_b$	$0.309 \pm 0.0031_c$	$0.296 \pm 0.034_d$
Heart rate (beat/min)	$274.50 \pm 13.4_a$	$243.80 \pm 7.1_b$	$243.00 \pm 5.8_b$	251.40 ± 7.4

$p<0.05$: different letters in the same line indicate significant differences.

The amplitudes and duration of P and T waves at three different times after ivermectin injection were statistically higher than those from the control group ($p<0.05$).

The comparison of the amplitude of the S waves showed that they were significantly ($p<0.05$) higher in the 24h-experimental group compared to controls. The S wave amplitudes measured 48 and 72 hours after injection were higher than that of the control group but the difference was not statistically significant.

The duration of PR and QTc intervals lengthened when examined at 24, 48 and 72 hours after ivermectin injections and were statistically longer ($p<0.05$) than that of the control group. In addition there were significant decreases ($p<0.05$) in the QTc duration recorded at 72 hours compared to those obtained at 24 and 48 hours.

Regarding QRS complex duration, statistically significant differences were seen only between the controls and those animals recorded at 24 and 48 hours after injection ($p<0.05$). The heart rate was significantly ($p<0.05$) decreased at 24 and 48 hours after ivermectin application compared to the control group.

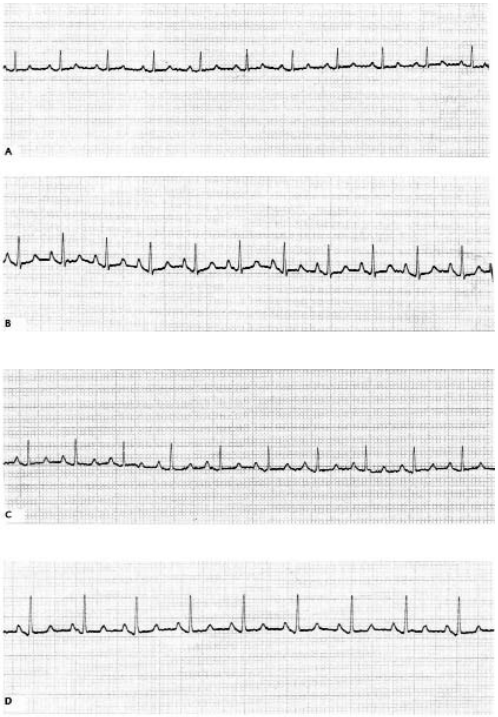

Figure 1. Representative electrocardiograms (limb leads II) of guinea pigs prior to treatment with ivermectin (A), and at 24 (B), 48 (C) and 72 hours (D) after treatment. (standardization, 1mV= 10 mm; chart speed, 50 mm/s).

Discussion

In this study, one single dose of 500 µg/kg b.w. ivermectin was administered subcutaneously to guinea pigs, as studied by McKellar *et al.* (*1992*) who were looking for the clinical and pharmacological characteristics of ivermectin. Recording time of the electrocardiograms was set at 24, 48 and 72 hours after injection since the maximum effectiveness of the drug in the plasma of guinea pigs was found to be at 72 hours (*Mc Kellar et al., 1992)*. However, the results here showed that significant differences in the electrocardiographic values were mainly seen at 24 and 48 hours after ivermectin injections. In addition, the reason for not using any anaesthetics in this research was their negative effects such as a lengthening in the duration of P wave, PR and QT intervals and a decrease in the duration of QRS complex (*Pişkin et al., 1999)*.

The electrocardiographic data obtained from the control group showed similar patterns to those published by Cieślar *et al*. (*1986*). The differences in the duration and amplitude of P and T waves observed at each of the three times after injection (24, 48 and 72h) compared to those of the control group meant the changes in illustrate transmission caused by the drug. The increase in amplitude and prolongation in duration of the P wave can be interpreted as having a strengthened systole and returning to the late depolarisation respectively. Moreover, it would be interesting to observe the changes in cellular Ca^{2+} level, which is regulated during normal contraction-relaxation cycle, especially in cardiac myocytes since they are considered

to play an important role in both mechanical dysfunction and arrythmogenesis associated with congestive heart failure (*Bers, 2000*). There have been various studies showing a decline in cardiac force due to a decreased Ca^{2+} sensitivity of the myofilaments and/or a deterioration of the Ca^{2+} signals (*Niggli, 1999*). During membrane depolarisation, Ca^{2+} flows across the membrane into the cardiac muscle fiber after being regeneratively released from the sarcoplasmic reticulum causing an increase in the concentration of free Ca^{2+} in the myoplasm (*Reuter, 1974*).

It can be suggested that, the inhibitory effect of ivermectin, like several macrocyclic lactone compounds (i.e. tacrolismus (FK506), ascomycin, rapamycin), on the sarcoplasmic reticulum Ca^{2+} - ATPase (SERCA) pump (*Ahern et al., 1999; Bultynck et al., 2000)* is a crucial point in explaining the changes of the contraction-relaxation cycle since SERCA was shown to be one of the most important transporters which remove Ca^{2+} from the cytosol (*Bers, 2000)*. Thus, the reason of the prolonged and enlarged depolarisation with a late Ca^{2+} accumulation after systole (*Frank et al., 2002)* might possibly be the result of the reduced amount of Ca^{2+} secondary to the decreased activation of SERCA. Similarly, the late loading of Ca^{2+} during systole would also be seen by the removing of Ca^{2+} during diastole (T wave), again because of the decreased activation of SERCA.

According to our results, vermectin significantly changed all ECG parameters after 24h except the R wave amplitude and this effect continued at 48h and 72h for most of them although they tended to revert to the control level McKellar *et* al. (*1992*) found that the level of ivermectin in the plasma was maximum at 72h and lower then in other species; they also mentioned that the differences in metabolism of ivermectin in guinea-pigs were either more rapid or slower than the others. Considering their measurement, which was only done at 72h for guinea-pigs, and other literature like Minematsu *et al.* (*1999, 2001*) showing that FK506, which is a macrocyclic lactone similar to ivermectin caused QTc lengthening and reached the maximum level at 90 mins. in whole blood and plasma as well as ventricular myocytes; however, it was decreased in plasma and whole blood rapidly while was continuing to effect the myocardium. This was probably the logical explanation of the sustained effect of ivermectin on some ECG parameters found in this study after 72h.

In this research, the lengthening of PR and QTc intervals and duration of P and T waves, and also the decrease in the heart rate, might be the indicator of the weak conductance (negatively dromotropic) depending on ivermectin-caused hyperpolarization by opening the chloride channels. There are several references showing various ivermectin receptors in mammalian and invertebrates (*Fisher & Helmut, 1992*). Opening the glutamate-gated Cl^- channels is the only certain known mechanism of ivermectin (*Cully et al., 1994*), although this can not be the mechanism seen here due to the absence of these channels in vertebrates (*Gerard et al., 2000*). Although its efficacy and easy use at different doses and routes in guinea pigs were reported by Webb *(1992*), its toxicity in the rodents (*Campbell, 1989*) and the negative effects of ivermectin on the heart of guinea pigs suggested in this research must be taken into consideration while evaluating its safety when using this drug even at treatment doses.

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