Evaluation of ECG Time Intervals in a Rabbit Model of Anthracycline-Induced Cardiomyopathy: A Useful Tool for Assessment of Cardioprotective Agents

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Received September 18, 2006
Accepted January 4, 2007

Summary
The aim of this study was to analyze the ECG time intervals in the course of the development of chronic anthracycline cardiomyopathy in rabbits. Furthermore, this approach was employed to study the effects of a model cardioprotective drug (dexrazoxane) and two novel iron chelating compounds - salicylaldehyde isonicotinoyl hydrazone (SIH) and pyridoxal 2-chlorobenzoyl hydrazone (o-108). Repeated daunorubicin administration induced a significant and progressive prolongation of the QRS complex commencing with the 8th week of administration. At the end of the study, we identified a significant correlation between QRS duration and the contractility index dP/dtmax (r = -0.81; P<0.001) as well as with the plasma concentrations of cardiac troponin T (r = 0.78; P<0.001). In contrast, no alterations in ECG time intervals were revealed in the groups co-treated with either dexrazoxane or both novel cardioprotective drugs (SIH, o-108). Hence, in this study, the QRS duration is for the first time shown as a parameter suitable for the non-invasive evaluation of the anthracycline cardiotoxicity and cardioprotective effects of both well established and investigated drugs. Moreover, our results strongly suggest that novel iron chelators (SIH and o-108) merit further study as promising cardioprotective drugs against anthracycline cardiotoxicity.

Key words
Anthracyclines • Daunorubicin • Dexrazoxane • Iron chelator • ECG • Troponin T

Since the late 1960s, anthracycline antibiotics have been among the most effective and widely used antineoplastic agents. Unfortunately, their repeated administration is accompanied by the risk of severe dose-dependent cardiotoxicity, which manifests itself as a left ventricular (LV) heart failure. Besides the typical abnormalities in LV contractility, the changes in cardiac electrophysiology have also been reported – mainly disturbances in ventricular depolarization and repolarization as well as a significant prolongation of the corresponding ECG intervals (van Acker et al. 1996, Noda et al. 1998).
As the chronic type of anthracycline cardiotoxicity represents a serious and largely irreversible complication of chemotherapy, great effort has been dedicated to prevent its onset. To date, numerous different substances have been studied with the aim to prevent or reduce the anthracycline-induced cardiomyopathy. However, at present only dexrazoxane (DXZ), a bisdiketopiperazine iron-chelating agent has been found to be sufficiently effective and has been approved for clinical use. A product of intracellular hydrolysis of DXZ (ADR-925) probably reduces anthracycline cardiotoxicity by binding free or loosely bound intracellular iron, and thereby it prevents the formation of highly toxic hydroxyl radicals (Malisza and Hasinoff 1996). As the use of dexrazoxane suffers from certain disadvantages, other alternative agents with potentially cardioprotective effects are currently under study – among others arolydhrazones iron chelators salicylaldehyde isonicotinoyl hydrazone (SIH) and pyridoxal 2-chlorobenzoyl hydrazone (o-108). Our previous studies have revealed no significant cardiotoxic side effects of these novel chelators as well as any changes of ECG (Adamcová et al. 2002, Šimůnek et al. 2005, Štěrba et al. 2005, 2006).

The present study aimed to analyze the changes in ECG intervals in the time course of the development of chronic anthracycline cardiotoxicity on a validated rabbit model (Geršl and Hrdina 1994, Šimůnek et al. 2004). Furthermore, this approach was employed to study cardioprotective effects of both the well established cardioprotectant (dexrazoxane) and two novel agents (SIH and o-108). The usefulness of ECG analysis in the experimental study of anthracycline cardiotoxicity has been indicated by various authors in different species. However, in rabbits there is the only study of Dzielska-Olczak et al. (2004), which assessed the ECG changes in doxorubicin-treated rabbits and concluded that ECG alone does not always reflect heart damage and therefore should not be used as a sole evidence of cardiotoxicity.

All experiments were performed under the supervision of the Ethical Committee of the Faculity of Medicine in Hradec Králové. The study was carried out on Chinchilla male rabbits. All agents were given once a week for 10 weeks in following groups:
- **control** (saline, 1 ml/kg i.v.), n = 11
- **DAU** (daunorubicin, Cérubidine, 3 mg/kg i.v.), n = 15
- **DAU + dexrazoxane** (Cardoxane, 60 mg/kg i.p., 30 min before each DAU), n = 5
- **DAU + SIH** (1 mg/kg i.v. in PEG 300/ethanol/saline, 10 min before each DAU), n = 8
- **DAU + o-108** (10 mg/kg i.p. in 10% Cremorphor EL, 30 min before each DAU), n = 8

The electrocardiograms (standard bipolar leads) were recorded and evaluated using the ADI PowerLab/8SP and software Chart for Windows v. 3.4.11 (ADI Instruments, Australia) at the beginning of the study and thereafter in selected time intervals (Fig. 1) before drug administration. Cardiac troponin T (cTnT) as a selective and sensitive marker of heart damage induced by chemotherapeutics (Adamcová et al. 1999, 2005) was determined in heparinized plasma using the Elecsys Troponin T STAT Immunoassay (Roche, Switzerland). All experiments were done under ketamine anesthesia (30 mg/kg i.m.). Invasive hemodynamic measurements of the left ventricular contractility index (dP/dt max) were performed at the end of experiment under pentobarbital anesthesia (30 mg/kg, i.v.). The left carotid artery was prepared and a PE catheter, filled-in with heparinized (10 IU/ml) saline was introduced into the left heart ventricle. After a 15-minute equilibration period, the measurement of maximal rate of the pressure rise in the isovolumic phase of the systole (dP/dt max) was performed using an ADI PowerLab 8SP apparatus (ADI Instruments, Australia) with appropriate transducers. Statistical evaluation was performed with the statistical software SigmaStat for Windows 3.0 (Jandel, Germany). Significances of differences were assessed with One-way ANOVA or Kruskal-Wallis ANOVA on Ranks. Spearman Rank Order Correlation was used to determine strengths of association between the QRS duration and cTnT or dP/dt max.

No significant changes either in the heart rate or the PQ interval were observed in the experimental group during the whole experiment. Surprisingly, we failed to find any consistent significant alteration of QT interval, which seemed to be the most promising parameter from the literature. This may result from difficulties accompanying precise determination of the end of repolarization on ECG of rabbits. Indeed some other authors have described similar experience with ECG in other species, e.g. with dog having a very labile T wave and the rat having the ST segment short or nonexistent (Gad 1992).

However, repeated DAU administration induced a significant prolongation of the QRS complex starting at the 8th week (0.043±0.001 s) as compared with both the initial value (0.039±0.001 s) and the control group (0.038±0.001 s) (Fig. 1). These findings corresponded well with other biochemical and functional parameters as
previously described (Geršl and Hrdina 1994, Adamcová et al. 1999), showing that critical cumulative dose of anthracycline is reached in our model during the 8th week. At the end of experiment, the prolongation of QRS in DAU group reached 120.5±3.9 % of the initial value, whereas in the control group only 103.5±2.6 % (p<0.05).

On the contrary, the same ECG analysis, performed at the end of experiment, did not reveal any significant alteration of QRS duration in the groups co-treated with either the well established cardioprotectant dexrazoxane (108.1±6.9 %; n.s.) or with our investigated compounds, i.e. SIH (104.8±4.6 %; n.s.) and o-108 (101.6±3.2 %; n.s.). The present findings are in accordance with other parameters under study, which is clearly shown by a good correlation between QRS complex and the left ventricular contractility index dP/dt\text{max} (r = –0.81; P<0.001), as well as with the plasma concentrations of cTnT (r = 0.78; P<0.001) measured at the end of the experiment (Fig. 2).

In agreement with the ECG data, the left ventricular contractility (dP/dt\text{max}) was significantly reduced in DAU-treated animals (783±53 kPa/s) compared to the control group (1345±61 kPa/s; P<0.05). Furthermore, co-treatment with cardioprotective drugs led to a significantly higher contractility than in the DAU group and no significant difference was observed in comparison with the control group: dexrazoxane (1411±77 kPa/s), o-108 (1131±124 kPa/s) and SIH (1184±80 kPa/s). In accordance with the ECG data, the last measured values of cTnT plasma levels during the experiment were significantly higher (P<0.05) in the DAU group (0.33±0.05 ng/ml) vs. controls (0.02±0.01 ng/ml). All groups with the cardioprotectants had the cTnT concentrations significantly lower (P<0.05) as compared with the DAU group – DXZ (0.04±0.02 μg/l), o-108 (0.21±0.03 μg/l) and SIH (0.22±0.05 μg/l).

We can therefore conclude that for the first time, the analysis of the QRS duration, performed on the validated model in rabbits, was found to be a suitable marker of chronic anthracycline cardiotoxicity. Moreover, it was shown that it is a useful parameter for simple evaluation of cardioprotective effects of new investigated agents. Hence, this study suggests that detailed analysis of electrical properties of the myocardium may be a valuable part of the preclinical evaluation of potential cardiotoxic as well as cardioprotective effects of drugs. Importantly, our findings further support the concept of intracellular iron chelation as a successful strategy for the protection against anthracycline cardiotoxicity (Swain and Vici 2004). Both SIH and o-108 were shown to be promising cardioprotective drugs which deserve further study.

**Acknowledgements**

This study was supported by the Research Project of MSM 0021620820.
References


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