Main title:

Early detection of anthracycline cardiotoxicity in a rabbit model: left ventricle filling pattern versus troponin T determination

Running title: Early detection of anthracycline cardiotoxicity in rabbits

M. ŠTĚRBA[1]✉, T. ŠIMŮNEK[2], O. POPELOVÁ[1], A. POTÁČOVÁ[3], M. ADAMCOVÁ[3], Y. MAZUROVÁ[4], M. HOLEČKOVÁ[5], V. GERŠL[1]

Charles University in Prague, Faculty of Medicine in Hradec Králové, [1]Department of Pharmacology, [3]Department of Physiology, [4]Department of Histology and Embryology and [2]Faculty of Pharmacy in Hradec Králové, Department of Biochemical Sciences and [5]University Teaching Hospital in Hradec Králové, Department of Clinical Biochemistry and Diagnostics, Czech Republic

✉ Corresponding author: Martin Štěrba, Charles University in Prague, Faculty of Medicine in Hradec Králové, Department of Pharmacology, Šimkova 870, 500 38 Hradec Králové 1, Czech Republic.

Tel.: +420 495 816 312; Fax: +420 495 518 509.

E-mail address: sterbam@lfhk.cuni.cz (M. Štěrba).

Summary

Anthracycline cardiotoxicity represents a serious risk of anticancer chemotherapy. The aim of the present pilot study was to explore and compare the potential of both the left ventricular (LV) filling pattern evaluation and cardiac troponin T (cTnT) plasma levels determination for the early detection of daunorubicin-induced cardiotoxicity in rabbits. The echocardiographic measurements of transmitral LV inflow as well as cTnT determinations were performed weekly for 10 weeks in daunorubicin (3 mg/kg weekly) and control groups (n=5, each). Surprisingly, no significant changes in LV-filling pattern were observed through the study, most likely due to the xylazine-containing anaesthesia, necessary for appropriate resolving of the E and A waves. In contrast, to the echographic measurement the dP/dt_{min} index obtained invasively at the end of the study revealed a significant impairment in LV relaxation, which was further supported by observed disturbances in myocardial collagen content and calcium homeostasis. However, at the same time cTnT plasma levels were progressively rising in the daunorubicin-treated animals from the 5th week (0.024±0.008 µg/l) until the end of experiment (0.186±0.055 µg/l). Therefore, in contrast to complicated non-invasive evaluation of diastolic function, cTnT is shown to be early and sensitive marker of anthracycline-induced cardiotoxicity in the rabbit model.

Keywords: daunorubicin, anthracyclines, cardiotoxicity, early detection, diastolic dysfunction, cardiac troponin T.

1. Introduction

Anthracycline antibiotics (e.g. doxorubicin, daunorubicin, epirubicin) are among the most effective chemotherapeutic agents in the treatment of both haematological and solid malignancies (Yee 2005 *et al*., Chabner 2006 *et al*.). Cardiotoxicity, however, represents a serious adverse reaction that largely limits their therapeutic potential and threatens the cardiac function of cancer survivors. All anthracycline derivatives may induce cardiac damage that can result even in life-threatening complication of chemotherapy. The main risk is associated with their chronic administration, when severe cardiomyopathy and heart failure may develop later in the course or any time after completion of the treatment (Von Hoff *et al*. 1979, Hrdina *et al*. 2000, Gharib and Burnett 2002).

As the anthracycline-induced cardiotoxicity is largely irreversible, it is crucial to detect the myocardial injury at its earliest possible stage. Among the first approaches employed for sensitive and reliable detection of anthracycline cardiotoxicity was the endocardial biopsy. However, its invasive nature hinders and in fact nearly prevents its routine use in seriously sick oncologic patients. Actual recommendations for cardiac monitoring of anthracycline-treated patients are mostly based on the non-invasive examination of the left ventricular (LV) systolic function, since its decline is a well-known hallmark of anthracycline cardiotoxicity (Alexander *et al*. 1979, Shan *et al*.1996, Tjeerdsma *et al*. 1999, Elbl *et al*. 2003, Elbl *et al*. 2005). Both echocardiography and radioventriculography are employed, however, relatively low sensitivity of these approaches do not allow covering the early phases of myocardial injury and therefore rather more pronounced and distinct cardiotoxicity can be revealed (Shan 1996 *et al*., Tjeerdsma *et al*. 1999, Suter *et al*. 2002).

While the effect of anthracyclines on the systolic function and its prognostic value is well established, the status of diastolic function, which actually may precede the impairment in contractility, remains to be clarified. As the diastolic function can be estimated noninvasively by Doppler echocardiography, its serial evaluation is well feasible. Several clinical studies dealing with the assessment of the diastolic function in anthracycline-treated patients were carried out and resulted in rather variable and in some cases also contradictive outcomes (Marchandise *et al*. 1989, Ewer *et al*. 1994, Bu'Lock *et al*. 1999, Bossi *et al*. 2001, Clements *et al*. 2002, Nousiainen *et al*. 2002, Elbl *et al*. 2003, Nakamae *et al*. 2004). However, these findings could potentially be affected by heterogeneity of patients (e.g. with respect to age, sex and anamnesis), dosage scheme, combination of chemotherapeutics, presence of chest irradiation, and the liquid load enhancing the excretion of metabolites. Interestingly, to our knowledge, there is no available data on the changes of the diastolic function in time obtained from a validated and well-reproducible experimental model of anthracycline-induced cardiomyopathy.

Apart from the examination of cardiac function, the modern advances in monitoring of chemotherapy-induced cardiotoxicity have brought the selective biochemical markers of cardiac injury into the focus. This approach theoretically might be of special value from the standpoint of early cardiotoxicity detection, since a low level release of myocardial biomolecules might be detected using sensitive analytical methods before any impairment of the heart function occurs (Petricoin *et al*. 2004). To date, probably the most interesting results have been described with cardiac troponins (Specchia *et al*. 2005, Adamcova *et al*. 2005, Lipshultz *et al*. 2004, Herman *et al*. 1998, Lipshultz *et al*. 1997) although other biochemical markers - e.g. natriuretic peptides - are also studied (Horacek *et al*. 2005, Koh *et al*. 2004). While both cardiac troponin T and I are at presence firmly settled as biomarkers in the diagnosis of myocardial infarction, their potential role in the early diagnosis of chemotherapy-induced cardiotoxicity and particularly in its early stages is not completely understood yet.

Several clinical as well as experimental studies have reported rise of cardiac troponins during or after the anthracycline treatment (Herman 1998, Adamcova 2005). Moreover, some authors suggest that even subtle early elevations of cardiac troponins in plasma of anthracycline treated patients might predict occurrence of chronic or delayed cardiotoxicity (Cardinale 2004). However, the exact value of elevated cardiac troponins for diagnosis and reliable prediction of chronic or late anthracycline cardiotoxicity is not well established yet and it deserves further studies. So far, most studies have used only few different selected time points for troponin T determination, which offer only limited information and does not allow to fully understand the role of troponin T as a biomarker during the whole anthracycline treatment. To date, there is a lack of systematic longitudinal study examining troponin T levels before and periodically through out the period of the development of chronic anthracycline cardiotoxicity.

The aim of the present pilot study was therefore to explore and compare the potential of both the LV-filling pattern evaluation and plasma troponin T determination for an early detection of daunorubicin-induced cardiotoxicity in rabbits.

2. Material and methods

2.1. Experimental animals

Fifteen Chinchilla male rabbits (3.5-4.5 kg at the beginning of the study) were used in this study. They were housed under a 12h light cycle, constant temperature and humidity. The animals had free access to water and a standard laboratory pellet diet. The animal experiments were performed in accordance with the “Guide for the care and use of laboratory animals“ (1996) and under the supervision of the Ethical Committee of the Medical Faculty in Hradec Králové.

2.2. Experimental design

In this study both the LV-filling pattern and plasma levels of troponin T were examined weekly in the daunorubicin and control groups (n=5, each) during 10 weeks. For appropriate evaluation of all parameters of the LV-filling the combined anaesthetic regimen (ketamine+xylazine) used by others for this purpose (Rungwerth *et al*. 2004; Nagueh *et al*. 2000; Litwin *et al*. 1994) had to be employed. However, since xylazine itself may have certain heamodynamic effects, another group of animals examined under ketamine alone (the ketamine group, n=5) was introduced to the study. The daunorubicin group was injected with daunorubicin (3 mg/kg, once weekly for 10 weeks) in a validated schedule for induction of daunorubicin cardiomyopathy (Gersl and Hrdina 1994; Simunek *et al*. 2004). Control and ketamine groups were receiving saline (2 ml/kg, once weekly, 10 weeks).

Blood for cardiac troponin T analysis was sampled weekly immediately after the Doppler measurements. The heart rate was determined from ECG. Body weight gain at the end of the study was determined. At the end of the experiment (i.e. 4 – 5 days after the last administration), invasive haemodynamic measurements were performed. The animals had been killed with pentobarbital overdose and their hearts were excised. The removed hearts underwent histological as well as biochemical examination.

2.3. Echocardiography

Since the echographic examination is unfeasible in conscious rabbits, the animals were anaesthetized with injectable anaesthetics. The ketamine group was anaesthetised with ketamine alone (50 mg/kg, i.m.), while in the control and daunorubicin groups combined anesthesia was used (ketamine 50 mg/kg + xylazine 2 mg/kg, i.m.). After the appropriate depth of anaesthesia was reached (approx. 10 min), the rabbits were placed on the table on the left lateral decubitus. The echocardiograms were obtained using a GE Vingmed CFM 800 apparatus (GE Vingmed Ultrasound, Norway) equipped with a 7.5 MHz standard paediatric probe. The apical four-chamber view was obtained by means of the left parasternal approach. The HPRF (High Pulse Repetition Frequency) mode of pulse-wave Doppler imagining was used to study the LV- filling. The mitral inflow was recorded through a sample volume of 1.2 mm placed at the tips of the mitral valve leaflets. Evaluated parameters (peak filling rates of E and A wave, acceleration and deceleration times) were determined from 3-5 cardiac cycles, while at least 3 different Doppler recordings were obtained during each examination.

2.4. Invasive haemodynamic measurements

In pentobarbital anaesthesia, the left carotid artery was prepared and a PE catheter (length 300 mm, inner diameter 1.0 mm), filled-in with heparinized (10 IU/ml) saline was introduced into the left heart ventricle. After a 15-minute equilibration period, the measurement of the following parameters was performed: the heart rate (HR), maximal rate of the pressure rise in the isovolumic phase of the systole (the maximum of the first derivative of LV pressure - dP/dt_{max}) and the maximal rate of pressure decline in the isovolumic phase of diastole (the minimum of the first derivative of LV pressure - dP/dt_{min}). Arterial blood pressure (BP) measurement was performed using another PE catheter inserted into the right femoral artery. An ADI PowerLab 8SP apparatus (Adinstruments, Australia) with appropriate transducers and the software CHART for Windows 3.4.11 were used for pressure measurements, their differentiation and recording.

2.5. Plasma levels of cardiac troponin T

Concentrations of cardiac troponin T (cTnT) were determined in heparinized plasma samples using an Elecsys Troponin T STAT Immunoassay on an Elecsys 2010 immunoassay analyzer (Roche, Switzerland) according to the manufacturer’s instructions. This third-generation assay is based on electrochemiluminescence immunoassay technology (ECLIA) using two mouse monoclonal antibodies in a sandwich format (two step assay). These antibodies show no cross reactivity with skeletal TnT for concentrations up to 1000 mg/ml (0.005%). The detection limit for the cTnT determination was 0.010 µg/l and values below this detection limit were considered to be zero.

2.6. Biochemical analysis of LV samples

Samples taken from the identical parts of the LV of the excised hearts were dried and after cooling period they were weighed and digested by microwave digestion with nitric acid and hydrogen peroxide. The total calcium content in the prepared samples was measured photometrically using flame photometry (Eppendorf, Efox 5053, Germany). Results are expressed as µg/g of dry tissue.

The collagenous proteins of the LV myocardium were determined as hydroxyproline concentration with an adjustment by factor 7.46 as previously described (Pelouch *et al.* 1995). Hydroxyproline concentration was measured as follows: homogenates of myocardial tissue (6.3%) were hydrolyzed with the equal volume of 4.2 mol/l NaOH at 120 °C for 20 min. Oxidation of the hydrolyzate (0.1 ml) was started with the addition of chloramine-T reagent (0.9 ml) at 25 °C and was stopped after 50 min by adding Ehrlich´s aldehyde reagent (1.0 ml). After incubation of samples at 65 °C for 20 minutes the absorbance of developed chromophore was read at 550 nm.

2.7. Histological examination

After macroscopic examination, the heart was dissected and the tissue blocks of the transversely sectioned left and right cardiac ventricles and left atrium (incl. auricle) were taken for histological examination. The material was fixed by immersion in 4 % formaldehyde for 5 – 7 days. Paraffin sections (6 µm thick) were stained with haematoxylineosin and Masson's blue trichrome. Photomicrographs were made with a Lucia G software version 4.51 (Laboratory Imaging, Prague, Czech Republic). A six-point scale (Score 0 - 5) for the semiquantitative evaluation of histopathological changes in the myocardium was used as previously described (Simunek *et al.* 2004).

2.8. Statistical analysis

All results are expressed as mean ± S.E.M. The statistical software SigmaStat for Windows 3.0 (Jandel, Germany) was used in this study. Significances of the differences were estimated using the One Way ANOVA unpaired test (comparison between groups) or the Paired t-test (comparison with the initial value within one group). The data without a normal distribution were evaluated using the nonparametric tests: Kruskal-Wallis ANOVA on Ranks and Wilcoxon Signed Rank Test.

3. Results

3.1. General toxicity

One premature death was observed in the daunorubicin group (in the 7th week). All animals in the control and ketamine groups survived until the end of the experiment. With respect to the initial body weights (control group = 4.04 ± 0.14 kg, daunorubicin group = 3.81 ± 0.11 kg, ketamine group = 4.04 ± 0.24 kg), a significant increase of this parameter was observed at the end of the study in the ketamine (111.54 ± 2.62 %, $p<0.05$) and control (117.18 ± 2.06 %, $p<0.05$) groups, while in the daunorubicin group it remained unchanged (98.97 ± 7.33 %, n.s.). The final body weights in the daunorubicin group were also statistically lower than those determined in the control group ($p<0.05$).

3.2. LV-filling pattern assessed by echocardiography

The parameters of transmitral inflow were evaluated serially weekly, for 10 weeks, in two groups of rabbits (daunorubicin and control) anaesthetised with the combined ketamine – xylazine regimen as well as in the ketamine group, where ketamine only anaesthesia was employed. The results are summarised in Fig. 2 (E-wave, A-wave, E/A) and Fig. 3 (acceleration time, deceleration time). No significant changes in any parameter were observed in the control group. Surprisingly, no significant trend in any parameter could be recognized also in the daunorubicin group, either in a comparison with the beginning of the experiment or in a comparison with the control group. Only few significant - however inconsistent - changes were observed in the daunorubicin group in comparison with the control group and no clear tendency could be distinguished. Unlike in control and daunorubicin groups, where typical biphasic filling was observed (Fig. 1B), only a one high peak was observed in ketamine group (Fig. 1A) - despite the maximal horizontal sweep setting. Therefore, here one could determine only the maximal peak-filling rate (E) and its acceleration and deceleration times. In comparison with both other groups, markedly different values were obtained in these parameters during the whole experiment, while only few other significant changes were noted (elevation of the maximal peak filling rate in the 7th week and a moderate decrease in deceleration time in the 7th and 8th weeks).

3.3. Invasive haemodynamic measurements

At the end of the study, the invasive haemodynamic measurements were performed. The parameters of the LV-relaxation (dP/dt_{min}) and contractility (dP/dt_{max}) are shown in Fig. 4. A significantly lower dP/dt_{min} was determined in the daunorubicin group in comparison with the control group. However, an even more prominent difference in dP/dt_{min} was apparent between the control and ketamine groups, which suggest a significant impact of xylazine on this parameter. Furthermore, Fig. 4 clearly demonstrates the significantly lower LV contractility (dP/dt_{max}) in the daunorubicin group as compared to the control group. Other haemodynamic parameters are summarised in Table 1. Although the mean heart rate was rather lower in the daunorubicin than in the control group, this did not reach statistical significance, whereas the difference in blood pressure was more prominent and significant. Importantly, both the latter parameters were significantly lower in the control group (combined anaesthesia) than in the ketamine group, which again points out the profound haemodynamic effect of xylazine.

3.4. Plasma levels of cardiac troponin T

Plasma cTnT levels in both the control and the ketamine groups were almost completely below the detection limit (i.e. <0.010 μg/l). Only a slight elevation above this limit was witnessed in few rare cases. In contrast, a marked progressive rise of cTnT levels was observed in the daunorubicin group commencing with the 3-4th week and reaching the statistical significance in the 5th week (Fig. 5).

3.5. Biochemical analysis of LV samples

The myocardial content of total calcium in the LV samples was determined to be 10.25 ± 1.36, 10.60 ± 0.67, and 15.79 ± 2.05 μg/g of dry tissue for ketamine, control, and danunorubicin groups, respectively. While very close values of total myocardial calcium were observed in the ketamine and control groups, a statistically significant elevation of total calcium concentration was found in the daunorubicin group.

The total amount of collagen (i.e. both soluble and insoluble) in the LV myocardium was significantly higher in the daunorubicin group (19.74 ± 0.79 mg/g wet weight) than in the control and ketamine groups (14.43 ± 0.37 and 12.95 ± 1.4 mg/g wet weight, respectively).

3.6. Histological examination

In the LV myocardium obtained at the end of experiment from the ketamine and control groups (Fig. 6 A,B), only weak signs of myocardial changes were detected. Only several scattered myocytes appeared to have increased eosinophilia of the cytoplasm, disintegration of myofibrils or pyknotic nuclei - Score 1. These changes were also observed in other parts of the heart wall without apparent local differences. On the contrary, a massive injury with the foci of necrotic cardiomyocytes, followed by a conspicuous macrophagic infiltrate (Score 3), and signs of progressive interstitial fibrosis (Score 4) were observed in the LV myocardium of all animals treated with daunorubicin (Fig. 6C). The most intensive morphological changes (particularly with respect to fibrosis) were observed in the LV free wall. Similar findings with somewhat lower incidence were also witnessed in the cardiac septum. The right ventricle wall was distinctly less damaged in this way. In the left atrium wall, the foci of scattered necrotic cardiomyocytes prevailed but no granular or fibrotic tissue was observed.

4. Discussion

Introduction of anthracycline antibiotics in the late 1960s represented one of the most important breakthroughs in modern oncology. Unfortunately, the risk of potentially fatal cardiomyopathy and heart failure remains to represent a major complication with potentially severe consequences. The early detection of anthracycline cardiomyopathy is therefore crucial for patient’s prognosis, since timely intervention (discontinuation of anthracycline treatment or indication of the cardioprotective agent - dexrazoxane) may allow preserving of both myocardial integrity and function.

In our previous experiments, we have demonstrated that repeated 10-week daunorubicin administration (3 mg/kg weekly) is able to induce marked cardiomyopathy and impairment of systolic function in rabbits. However, the decline in the LV ejection fraction was significant only by the end of the 10-week experiment (Simunek *et al*. 2004). These findings correspond with rather lower sensitivity of the echographically-estimated ejection fraction (Shan *et al*. 1996). Therefore, the present study was designed to systematically investigate and compare the feasibility and utility of two independent approaches (echographically examined LV filling pattern and plasma levels of troponin T) for early determination of anthracycline-induced cardiotoxicity in rabbits. In this study, we show that relatively high heart rate (though physiological in this species) makes Doppler evaluation of the LV-filling troublesome. As we also attempted to perform the Doppler measurements using other anaesthetics (e.g. a benzodiazepine midazolam) instead of ketamine without any improvement, wrong anesthesia cannot be blamed from this complication (unpublished results). In contrast, combinational anaesthetic regimen containing xylazine improved significantly the quality of Doppler recordings of transmitral inflow and the typical biphasic pattern has been reached (Fig. 1B). This improved quality of the recordings was obtained only at the expense of profound changes in the heart rate as well as other key cardiovascular variables (Table 1). Interestingly, this combination of injectable anaesthetics is, indeed, quite frequently used in experimental research and it can be also found in numerous echocardiographic studies performed on rabbits, rats and mice (Rungwerth *et al*. 2004; Bull *et al*. 2003; Derumeaux *et al*. 2002; Chaves *et al*. 2001; Teraoka *et al*. 2000; Tokudome *et al*. 2000; Nagueh *et al*. 2000; Pennock *et al*. 1997; Hoit *et al*. 1995; Litwin *et al*. 1994; Young *et al*. 1990).

In this study, we followed for the first time the LV-filling pattern in animals treated with daunorubicin in a standard schedule used for the induction of anthracycline cardiomyopathy. Surprisingly, no significant consistent changes in any parameter evaluated (E, A, E/A, acceleration time, deceleration time) could be observed during the whole study.

With few rare exceptions, these values were similar to those in the control group. These results are also comparable to those referred for control groups in the literature (Rungwerth *et al*. 2004; Nagueh *et al*. 2000). Despite of the negative findings of the echographic part of this study, the diastolic dysfunction was clearly proved by more sensitive invasive measurement performed at the end of the study - the dP/dt_{min} index (maximal rate of pressure decline in the isovolumic phase of diastole) was significantly reduced (Fig. 4). The invasiveness of this approach, however, markedly complicates the practical employment of this method and therefore its potential repeated use in clinical setting is, indeed, virtually unfeasible. The discrepancy observed between the non-invasive and invasive evaluation of the LV diastolic function in the present experiment is supposed to be likely related to the lower sensitivity and specificity of the Doppler measurement of the LV-filling together with the confounding effects of xylazine-containing anaesthesia, which significantly altered the heamodynamic parameters.

Our previous findings (Simunek *et al*. 2004) as well as other indices from this study strongly support the validity of the results of invasive measurements pointing out the significant abnormality in the diastolic function. The impairment in lusitropic properties of LV is known to be dependent on both myocyte component (represented mainly by calcium handling abnormality) and non-myocyte component (connective tissue proliferation creating the basis for myocardial fibrosis) (Katz 2001). In the present study, the biochemical analysis of LV samples obtained from daunorubicin treated animals revealed both significant impairment of total myocardial calcium content and marked elevation of the amount of collagenous proteins, which was also documented histologically (Fig. 6). Other morphological findings well documented typical left ventricular myocardial injury induced by chronic anthracycline treatment and are well in line with previous reports (Herman 1985, Simunek 2004).

In contrast to numerous complications experienced with serial evaluations of the LV-filling pattern, the troponin T plasma level determination is shown to be easy to obtain and a very sensitive marker of daunorubicin-induced cardiotoxicity. These experiments were first to systematically study the elevations of troponin T in plasma through out the whole course of development of anthracycline cardiotoxicity. We have experienced detectable elevations above the detection limit already since the 3-4th week (i.e. at cumulative dose of daunorubicin ≈ 100-150 mg/m^2), while commencing with the 5th week, the rise of this marker became statistically significant (cumulative dose of daunorubicin ≈ 200 mg/m^2). The progressive increase in cTnT levels well corresponds with the successive nature of chronic anthracycline cardiotoxicity, where the cardiac risk depends on the cumulative dose (von Hoffa *et al.* 1979). Our results thus suggest that - at least in our experimental conditions - troponin T is the earliest and very useful marker of cardiac toxicity induced by daunorubicin. Moreover, troponin T is a marker which may find applicability in other laboratory animal species (e.g. mouse, rat) employed for the modelling of anthracycline cardiotoxicity and evaluation of potential cardioprotectants (Wallace *et al*. 2004, Adamcova *et al*. 2005) and, indeed, it can be also used in the clinical practice.

In conclusion, using the standard echocardiography equipment, Doppler evaluation of the LV-filling pattern in rabbits seems to be complicated within the physiological heart rate. Xylazine-containing anaesthesia enables high quality evaluation of diastolic filling in this species; this approach, however, does not have a predictive value for the development of myocardial damage induced by daunorubicin. In contrast to the Doppler assessment of diastolic function, cardiac troponin T was shown to be a sensitive marker of early daunorubicin cardiotoxicity, which may be used in different animal species as well as in clinical practice. Therefore, cTnT deserves further experimental as well as clinical research as a biomarker of anthracycline-induced cardiotoxicity.

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Table 1 Heart rate and blood pressure at the end of the study

	HR (min^{-1})	**BP** (mmHg)
ketamine	298 ± 8	96 ± 6
control	206 ± 14 k	75 ± 8 k
daunorubicin	178 ± 23	51 ± 2 c

HR = heart rate, BP = mean arterial blood pressure. Statistical significance (ANOVA, $p<0.05$) in comparison with the control group - “c” and the ketamine group – “k”.

Figure legends

Fig. 1: Left ventricular transmitral inflow in rabbits – anaesthetized with a) ketamine 50 mg/kg or b) a combination of ketamine 50 mg/kg and xylazine 2 mg/kg. "E" – early (passive) and "A" – latter (active) component of the left ventricular filling.

Fig. 2: Peak filling rates of the passive and active components of the left ventricular filling and their ratio during the experiment. "E – wave" - early, passive component, "A – wave" - latter, active component, and E/A - ratio of both peak filling rates. Statistical significance – "*" in comparison with the initial values within each group (paired t-test, $p<0.05$) and "c" with the control group (ANOVA, $p<0.05$).

Fig. 3: Acceleration and deceleration times during the experiment. Statistical significance – "*" in comparison with the initial values within each group (paired t-test, $p<0.05$) and "c" with the control group (ANOVA, $p<0.05$).

Fig. 4: Maximal rate of pressure decline in the isovolumic phase of diastole (dP/dt_{min}) and maximal rate of the pressure rise in the isovolumic phase of the systole (dP/dt_{max}) determined invasively at the end of the study**.** Statistical significance (ANOVA, $p<0.05$), "n.s." – not significant.

Fig. 5: Cardiac troponin T plasma concentrations in the time-course of the study. Statistical significance – "*" in comparison with the initial values within each group (paired t-test, $p<0.05$) and "c", "k" with the control group and ketamine group (ANOVA, $p<0.05$), respectively.

Fig. 6: Histological examination of the left ventricular myocardium.

In both saline-receiving groups: ketamine (Fig A) and control (Fig B), increased eosinophilia of cytoplasm (E – homogenously eosinophilic cells; X – eosinophilic strips in the cytoplasm of myocytes), woven myocytes (W) and some scattered pyknotic nuclei (arrows) are the only present changes (score 1). C – normal cardiomyocytes. In a sharp contrast, repeated administration of daunorubicin (Fig C) causes a massive injury of the myocardium (score 4). The number of intensely eosinophilic cells (E) decreases whereas the foci of necrotic (N) cardiomyocytes, followed by a conspicuous macrophagic infiltrate (M), markedly increase in size and number. Healing process, i.e. the progressive interstitial fibrosis subsequently develops in this group. At first, the granulation tissue appears that mature to fibrotic tissue (marked by bundles of collagen fibers – F1). Finally, fibrotic scars of different size may also develop (F2). Masson's blue trichrome. Bar 30 μm.

Fig. 1

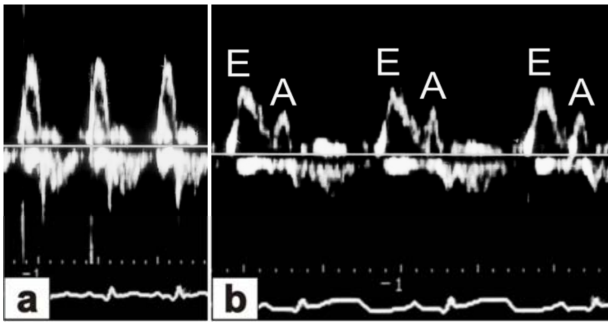
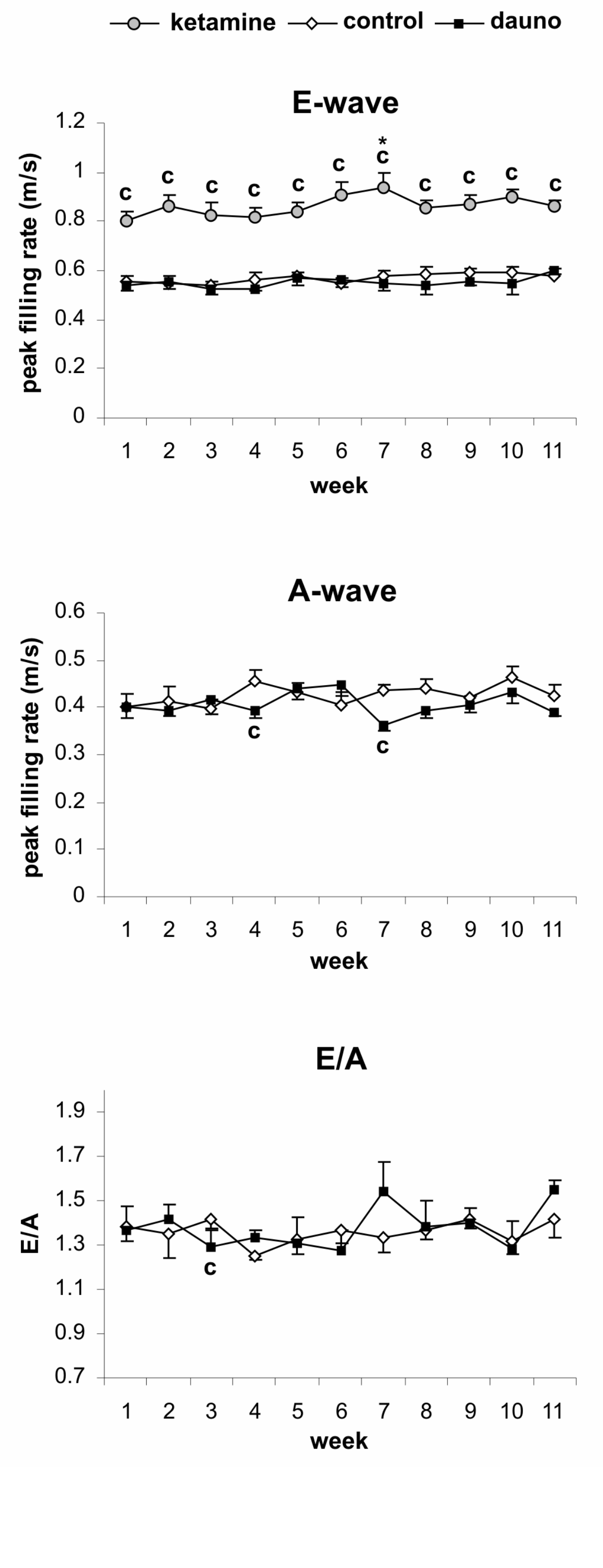
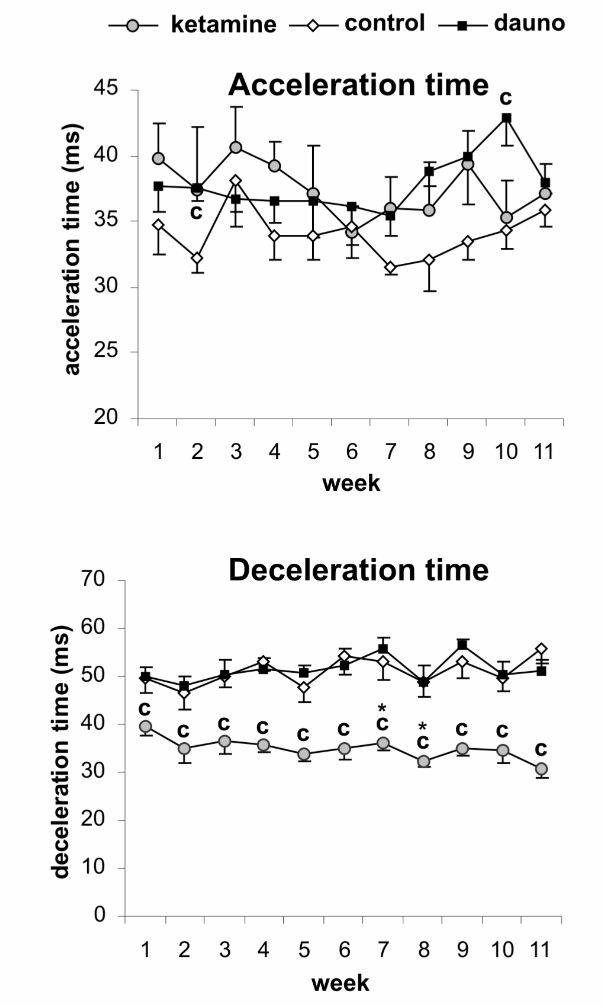


Fig. 2



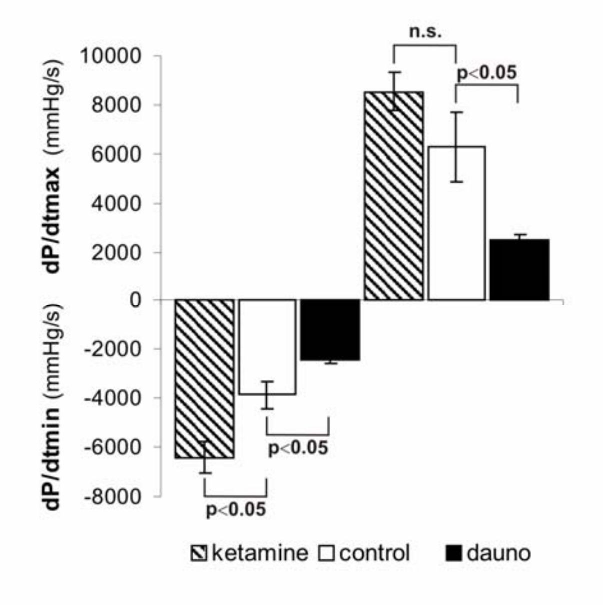
ketamine
control
dauno
E-wave
peak filling rate (m/s)
1.2
1
0.8
0.6
0.4
0.2
0
c
c
c
c
c
c
*
c
c
c
c
c
1 2 3 4 5 6 7 8 9 10 11
week
A-wave
peak filling rate (m/s)
0.6
0.5
0.4
0.3
0.2
0.1
0
c
c
1 2 3 4 5 6 7 8 9 10 11
week
E/A
E/A
1.9
1.7
1.5
1.3
1.1
0.9
0.7
c
1 2 3 4 5 6 7 8 9 10 11
week

Fig. 3



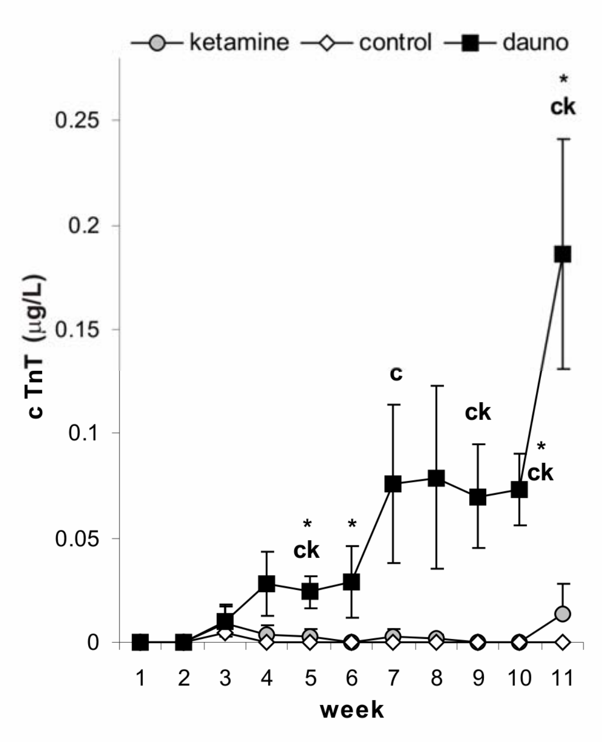
ketamine
control
dauno
Acceleration time
acceleration time (ms)
45
40
35
30
25
20
c
c
1 2 3 4 5 6 7 8 9 10 11
week
Deceleration time
deceleration time (ms)
70
60
50
40
30
20
10
0
c c c c c c * c * c c c c
1 2 3 4 5 6 7 8 9 10 11
week

Fig. 4



n.s.
p<0.05
10000
8000
6000
4000
2000
0
-2000
-4000
-6000
-8000
dP/dtmax (mmHg/s)
dP/dtmin (mmHg/s)
p<0.05
p<0.05
ketamine
control
dauno

Fig. 5



ketamine
control
dauno
c TnT (μg/L)
0.25
0.2
0.15
0.1
0.05
0
1 2 3 4 5 6 7 8 9 10 11
week
*
ck
c
ck
*
ck
*
ck
*

Fig. 6