



The cardiac effects of carbon nanotubes in rat

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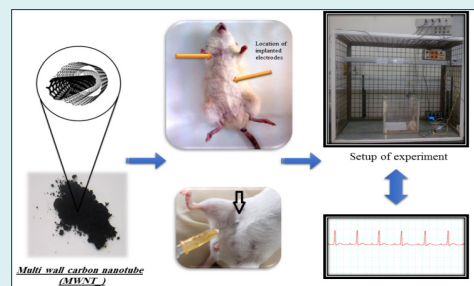
Abstract

Introduction: Carbon nanotubes (CNTs) are novel candidates in nanotechnology with a variety of increasing applications in medicine and biology. Therefore the investigation of nanomaterials' biocompatibility can be an important topic. The aim of present study was to investigate the CNTs impact on cardiac heart rate among rats.

Methods: Electrocardiogram (ECG) signals were recorded before and after injection of CNTs on a group with six rats. The heart rate variability (HRV) analysis was used for signals analysis. The rhythm-to-rhythm (RR) intervals in HRV method were computed and features of signals in time and frequency domains were extracted before and after injection.

Results: Results of the HRV analysis showed that CNTs increased the heart rate but generally these nanomaterials did not cause serious problem in autonomic nervous system (ANS) normal activities.

Conclusion: Injection of CNTs in rats resulted in increase of heart rate. The reason of phenomenon is that multiwall CNTs may block potassium channels. The suppressed and inhibited I_K and potassium channels lead to increase of heart rate.



Introduction

Carbon nanotubes (CNTs) with their special properties are regarded as unique materials in various health fields such as biomedicine and pharmacology.¹ Recently, the potential application of CNTs in drug and gene delivery has been clearly demonstrated (e. g. drugs are encapsulated in CNTs, and then they are injected into cell and are released there).² Furthermore, CNTs are promising candidates for forming the basis of new biological and medical devices such as pace makers and biosensors.³ For example, the CNT-based electrochemical biosensors have been used for detecting chemical redox interactions.^{4,5} Additionally, CNTs are used as scaffolds to promote cell adhesion, growth, differentiation, and proliferation. These scaffolds provide mechanical strength, chemical stability, and biological inertness.⁵ Another application of CNTs can be intracellular and extracellular recording.^{6,7} The benefits and the utilizations of CNTs may raise concerns about their biocompatibility and possible adverse effects on cardiovascular system.⁸ They may alter the au-

tonomic nervous system (ANS) and spontaneous heart rate variability (HRV).⁸ According to inhalation studies on particles, it has been predicted that nano-sized particles will have higher pulmonary depositions and biological activities compared with larger particles. Therefore, some nanomaterials may affect the deposition site and distant responses throughout the body.⁹ In general, the cytotoxicity of CNTs depends on their structure, dose, concentration, manufacturing method, functional group, etc.¹⁰ Several in vitro and in vivo studies demonstrated that CNTs might induce oxidative stress and cytotoxic effects.¹⁰ These nanomaterials may cause pulmonary inflammation.¹⁰ The investigation of cardiovascular adverse effects of single-wall carbon nanotubes (SWCNTs) on respiratory exposure shows that they lead to formation of pulmonary granulomatous and production of cardiovascular toxicity.¹¹ A toxicity investigation of multi-walled carbon nanotubes (MWCNTs) in human shows that not only they induce inflammatory and fibrotic reactions but also they lead to protein exudation and



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granulomas on the peritoneal side of the diaphragm.¹² One of the in vitro studies about CNTs cardiovascular effects showed that exposure to CNTs increase the risk of cardiovascular diseases by affecting normal cardiac electrophysiology.¹³

Another study shows that oropharyngeal aspiration of MWCNT causes increased susceptibility of cardiac tissue to ischemia/reperfusion injury without a significant pulmonary inflammatory response; therefore MWCNTs can be the cardiovascular system risk.¹⁴ Generally, there is concern that nanomaterials could have a major impact on the cardiovascular system, although the effects of exposure to newly developed nanomaterials on the cardiovascular system remain elusive and no definitive data are available about these nanomaterials' effect on the cardiovascular autonomic control.^{8,15} Inhaled nanoparticles in the lungs may cause systematic inflammation through oxidative stress, which mediates endothelial dysfunction and atherosclerosis. In addition, nanoparticles can translocate into the blood stream, be taken up by endothelial cells, and directly induce injury of endothelial cells.¹⁵

Among all previous works, there are rarely studies about the cardiac effect of MWCNTs in rats during electrocardiogram (ECG) analysis. As it was mentioned earlier, the aim of present study was to investigate the CNTs' effect on the heart of rats. The ECG represents the cardiac electrical activity. A typical ECG tracing is a repeating cycle of three electrical entities: a P wave (atrial depolarization), a QRS complex (ventricular depolarization), and a T wave (ventricular repolarization). They are measured by electrodes in typical engagement.¹⁶ ECG analysis represents the heart function, and changes during a kind of activity or the state of ANS are evaluated by means of ECG analysis. Therefore, to investigate the biocompatibility of CNTs in ANS activity, the ECG signals are considered. Recording is done before and after injection of CNTs. Time and frequency domain features for analyzing ECG signals were used in terms of HRV. The rhythm-to-rhythm (RR) intervals in HRV method were computed and features of signals in time and frequency domains were extracted pre- and post-injection. Finally results were discussed and the conclusion was done.

Materials and methods

Characteristics of animals

The experiment was carried out on male Wistar rats weighing 280 g to 300 g and each group included six rats. Animals were housed in standard polpropylene cages, three per cage, under a 12:12 h light/dark cycle at an ambient temperature of $22 \pm 2^\circ\text{C}$ and had free access to food and water. All experiments were carried out under ethical guidelines of Tabriz University of Medical Sciences, for the care and use of laboratory animals (National Institutes of Health Publication No 85-23, revised 1985).

Chemical materials

For this study, functionalized MWCNTs with carboxyl group were used. They were purchased from Neutrino

Company (Tehran, Iran). These functionalized MWCNTs were in solid powder and black color. COOH content of this material was 2 wt %. They had an inner diameter of 5 to 10 nm, an outer diameter of 10 to 20 nm, and an individual length of $\sim 30 \mu\text{m}$ according to the manufacturer. Their purity was more than 95 wt %.

Surgical procedure

Rats were anaesthetized with injection of ketamine (80 mg/kg, i.p) and xylazine (20 mg/kg, i.p.). For ECG recording, two electrodes were implanted under the skin and ECG signal was acquired from lead II (voltage between the right arm and the left leg). The ECG electrodes (BP disposable chest electrodes) were soldered to copper wires. To prevent the wire damaging by the rat, they were conducted subcutaneously to back of the body.

Injection and ECG recording

Three days after the surgery, animals were settled in Faraday cage in a freely moving condition for ECG signal recording. In electrophysiology, it is a very common method that the experimentalist records EEG or ECG signals for a few minutes before drug injection, then drug injection is performed and the recording is continued. After these stages, the recorded data from before and after drug injection is analyzed and compared with each other. Since the injection of saline as control group had no significant effect on EEG and ECG recording in pervious papers of our college,¹⁷⁻¹⁹ we used our prior data about the effect of saline injection. Therefore, we had no additional group for saline injection as control; the group before injection of CNT was considered as control group.

As mentioned above, the ECG signals were recorded for one hour, before CNT injection (as control group) and after it. For intra-peritoneal injecting of CNTs, 1 mg/kg body weight corresponding to 0.3 mg/rat of MWCNTs was considered. MWCNTs was dissolved in 0.9% saline for suspension and prepared at a concentration of 0.5 mg/mL and then it was injected to the rats. Half an hour after injection, ECG signals were recorded again for each rat. Fig. 1 shows an arbitrary sample of ECG recording, before and after CNT injection for 2 s from 11:05 to 11:07 (min: s).

ECG signal analysis

The HRV analysis method is used for ECG signals analysis. Before extraction features for signal analysis, a 45 Hz low pass filter was used for removing noise from the ECG data. HRV analysis was developed in order to assess ortho-sympathetic and parasympathetic influences.²⁰ Analyzing HRV is a powerful tool to evaluate and investigate cardiac heart rate.^{21,22} The interval between adjacent QRS complexes in ECG signal is termed as the RR interval. The variation of RR interval is referred to be as the HRV.²³ Each R peak, due to sinus depolarization, was detected from a continuous ECG signal, and the normal RR intervals were determined. Then they were analyzed in time and frequency domains. In the time domain analysis, the

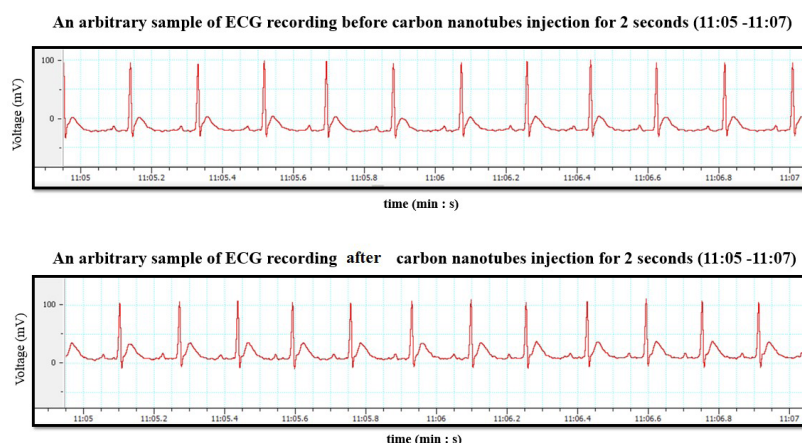


Fig. 1. An arbitrary sample of ECG recording before and after CNT injection for 2 s, from 11:05 to 11:07 (min : s). (Top row) before CNT injection, (bottom row) after CNT injection.

intervals between adjacent normal R peaks were measured over the period of recording. A variety of statistical parameters were calculated from the intervals.²⁴

In frequency domain, Welch’s method was considered to calculate the power spectral density (PSD) of RR intervals.²⁵ One of the important statistical parameters in frequency domain was the ratio of power in the low-frequencies (LFs) to the power in high frequencies (HFs). Study of HRV in time and frequency domains were done for the RR intervals of rats former and later to injection of CNTs.

Time domain analysis

Time domain analysis of selected RR included mean of RR interval series RR_{mean} , standard deviation of all NN intervals (SDNN), the root mean square of differences of successive RR intervals (RMSSD), and mean heart rate (HR_{mean}). There were N numbers of RR intervals in a segment.

Frequency domain analysis

HRV displays two main frequency components that represent ANS activity: a LF component which demonstrates both sympathetic and vagal activation and a HF component that indicates parasympathetic activity. The higher power of LF indicates high stress and the higher power of HF indicates low stress. The LF/HF ratio or the fractional LF power has been used to describe sympathovagal balance.²⁶ Welch’s method is used for determining PSD of signals. The LF and HF frequency bands of the RR intervals were set at 0.4–1.5 Hz and 1.5– 5 Hz, respectively. The power of LF and HF frequency bands was computed using Welch’s method, then LF_{norm} and HF_{norm} were calculated which is as Eqs.1 and 2:

$$LF_{norm} = \frac{LF}{LF + HF} \quad (1)$$

$$HF_{norm} = \frac{HF}{LF + HF} \quad (2)$$

The LF_{norm} indicates the LF power and the HF_{norm} shows the HF power in normalized unit. The sympathovagal balance (LF_{norm} / HF_{norm} ratio) is computed through dividing

Eq.1 by Eq.2.

Statistical analysis

In this study, for statistical analysis, the SPSS software was used. To test the differences in behavioral experiment, the paired sample *t* test was done on results of HRV analysis.

Results and Discussion

Results of ECG analysis using HRV method

HRV features in time and frequency domains were calculated for RR intervals. Table 1 shows $LF_{norm_{a_i}} / HF_{norm_{a_i}}$ and $LF_{norm_{b_i}} / HF_{norm_{b_i}}$ ratios for a_i and b_i cases (the “after” and “before injection” words are indicated by a_i and b_i indexes, respectively).

According to Table 1, it was seen that for RR intervals, the mean of $LF_{norm_{a_i}}$ and $LF_{norm_{a_i}} / HF_{norm_{a_i}}$ ratio were lower than $LF_{norm_{b_i}}$ and $LF_{norm_{b_i}} / HF_{norm_{b_i}}$ ratio, respectively. On the other hand, the mean of $HF_{norm_{a_i}}$ was higher than $HF_{norm_{b_i}}$, though differences for all of them were not significant ($p > 0.05$). These results showed that CNTs were not change sympathovagal balance and therefore these nanomaterials did not make any problem in ANS activity. The results of RR intervals analysis in the time domain is summarized in Table 2.

In Table 2, the results of RR intervals analysis are presented for CNT injection group (a_i) and control group (b_i). The results of before and after injection for these two factors showed that the values of $SDNN_{a_i}$ are lower than $SDNN_{b_i}$ and $RMSSD_{a_i}$ are higher than $RMSSD_{b_i}$. The differences of these factors were not significant ($p > 0.05$ for both). The differences of mean values were more than 10^{-2} , but because of restriction of software, values were rounded up to 2 correct significant digits; therefore, Table 2 shows that the mean values of $SDNN_{a_i}$ and $SDNN_{b_i}$ and also $RMSSD_{a_i}$ and $RMSSD_{b_i}$ were equal. In RMSSD results, it was seen that CNTs did not significantly increase the stress. The result of RR_{mean} analysis was significant ($p = 0.004$). These results for a group of rats including former and later to injection of CNTs are shown in Fig. 2.

As it is shown in Fig. 2, the value of RR_{meana_i} was higher

Table 1. Paired samples statistics for frequency domain features

Name	Mean	Number	SD	P
HF _{norm} *	HF _{normb_i} 0.84	6	0.133	0.457
	HF _{norma_i} 0.88	6	0.105	
LF _{norm} **	LF _{normb_i} 0.16	6	0.133	0.475
	LF _{norma_i} 0.12	6	0.105	
LF _{norm} /HF _{norm}	LF _{normb_i} /HF _{normb_i} 0.23	6	0.261	0.484
	LF _{norma_i} /HF _{norma_i} 0.15	6	0.151	

*High-frequency; ** Low-frequency.

Norm is pointed to normal time.

The “after” and “before injection” words are indicated by a_i and b_i indexes, respectively.

Table 2. Paired samples statistics for time domain features

Name	Mean	Number	SD	P
HR _{mean} *	HR _{meanb_i} 360.13	6	30.292	0.062
	HR _{meana_i} 323.61	6	26.267	
RR _{mean} **	RR _{meanb_i} 0.17	6	0.014	0.004
	RR _{meana_i} 0.19	6	0.011	
RMSSD***	RMSSD _{b_i} 0.01	6	0.002	0.338
	RMSSD _{a_i} 0.01	6	0.004	
SDNN****	SDNN _{b_i} 0.01	6	0.002	0.758
	SDNN _{a_i} 0.01	6	0.003	

* Mean heart rate; ** Mean of RR interval series; *** The root mean square of differences of successive RR intervals.

than RR_{meanb_i}. The p value for HR_{mean} was 0.062 (this value was lower than 0.1, hence it was considered significant). These results for a group of rats including former and later to injection of CNTs are shown in Fig. 3.

As it is shown in Fig. 3, the value of HR_{meanb_i} was higher than HR_{meana_i} and the value of HR_{mean} for rats was increased after injection. Analysis of these features showed that injection of CNTs increased the numbers of heart beats.

CNTs, according to their electrical, chemical, optical, mechanical, and thermal properties, have different applications in medicine, including the application in drug and gene delivery, as substrates or scaffolds for neuronal growth and so on. But the application of CNTs in medicine is closely dependent on their biocompatibility with body system.²⁷ There are a few reports about biocompatibility of these nanomaterials.

Initial toxicological studies demonstrated that pharyngeal installation of SWCNT suspension in mice caused a persistent accumulation of CNT aggregates in the lung. It is followed by the rapid formation of pulmonary granulomatous, functional respiratory deficiencies and fibrotic tissues at the site.^{28,29} Some animal studies demonstrated that inflammatory and granulomatous responses are seen in the lung following exposure to CNTs.²⁹⁻³¹ Different surfactants affect biocompatibility of CNTs. Investigation of the cytotoxicity of CNT dispersed in different surfactants showed that cytotoxicity of CNTs depends on surfactant selection; for example, SDBS (Sodium dodecylbenzene-

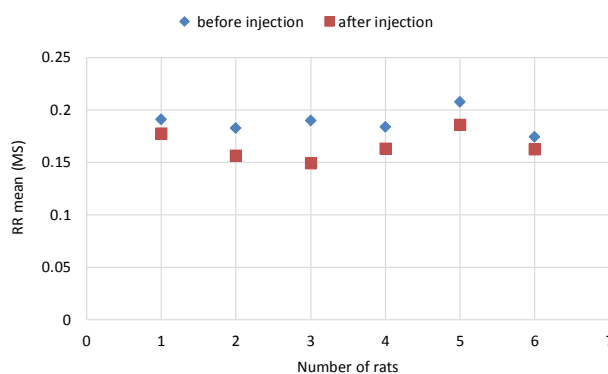


Fig. 2. RR_{mean} plot for each rat (** p = 0.004). It is shown that the mean of RR intervals measures is decreased after injection.

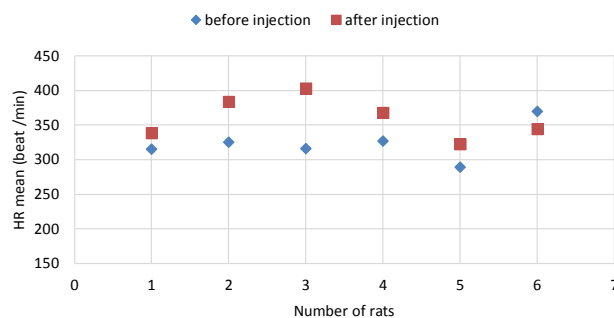


Fig. 3. HR_{mean} plot for each rat (* p = 0.062). It is shown that the heart rate or the number of beats is decreased after injection.

sulfonate) led to toxicity and SC (sodium cholate) did not cause toxicity.^{32,33} Studying the effect of functionalized MWCNTs by -COOH on the potassium (K⁺) channels (I_{to}, I_K, I_{K1}) showed that MWCNTs suppress the K⁺ channels activity. On the other hand, Suppression of potassium channels did not associate with an induction of oxidative stress.³⁴ The CNTs blocked the potassium channel pore and interrupted ion permeability. Similarity, CNTs caused a significant impairment in cytoplasm elevation when neurons were depolarized; this may be due to CNTs interfering with the function of channels.³⁵ Recent study about effect of CNTs in cardiovascular system shows that pulmonary instillation MWCNT has the potential to cause cardiovascular abnormality. This study demonstrated that pulmonary exposure to MWCNT can be manifested as a reduced epithelial barrier and activator of vascular gp130-associated transsignaling; however the mechanism of CNT's effects are unclear.³⁶ In vitro tests investigation showed that MWCNTs were biocompatible, and no damage at the cellular structural level was observed.³⁷ According to one of previous works, injection of MWCNTs led to a transient and self-limiting local inflammatory response.³⁸ Generally, MWCNTs were reported to be more biocompatible than SWCNTs; therefore, they had more utility than SWCNTs for medical applications.³⁹ As we know, potassium has the most important intracellular action, and it has a major role in determining the resting membrane potential of cells. Changes in potassium

gradient across the cell membrane can result in cell dysfunction. This may mostly affect cardiovascular, resulting in cardiac arrhythmias. Potassium concentration changes in serum may occur as hyperkalemia and hypokalemia. Hypokalemia leads to shifting the cell resting membrane potential towards positive values and prolong the potential duration of the action and refractory period which are potentially arrhythmogenic.⁴⁰ Hypokalemia increases the risk of developing ventricular arrhythmias such as ventricular tachycardia post myocardial infarction ventricular ectopic beats, and ventricular fibrillation.⁴¹ Hyperkalemia results in loss of resting membrane potential and reduction of potential action duration. When extracellular K^+ was raised to levels around 15 mM, ventricular fibrillation occurred.⁴²

The results of this study showed that injection of CNT increases the heart rate. It is obtained by investigating the results of two factors including HR_{mean} and RR_{mean} . The MWCNTs could be a potassium channel blocker. The mechanism that was involved in increasing the heart rate was probably suppressed and potassium channels was inhibited by MWCNTs. They led to a change in activating potential of mentioned potassium channels in direction of depolarization.

Conclusion

There is a potential concern about biocompatibility of CNTs in body function. Cardiac effect of MWCNTs in rat was studied in this paper. Effects of MWCNTs on ECG signals in one group of rats were investigated former and later to injection of CNTs. Analyses of the RR interval variability for ECG signals were done former and later to injection in time and frequency domains. According to the results obtained in frequency domain, MWCNTs did not affect the sympathovagal balance significantly. On the other hand, the results of HRV method in time domain showed that injection of CNTs increased the heart rate. The reason for this phenomenon was the possible inhibition of I_{to} , I_{Kr} , and I_{K1} potassium channels by MWCNTs. In general, nanotubes do not cause serious problem in ANS normal activities, however they are not completely biocompatible on the heart.

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Ethical approval

There is none to be declared.

Competing interests

The authors declare no conflict of interests.

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Study Highlights

What is current knowledge?

- ✓ CNTs with their special properties have the potential application in biomedicine, pharmacology, and neurosciences.
- ✓ There is a potential concern about biocompatibility of CNTs in body function.
- ✓ The CNTs' impact in ANS is investigated by ECG signals analysis.

What is new here?

- ✓ CNTs do not affect the sympathovagal balance significantly.
- ✓ Injection of CNTs into rats' body resulted in increase of heart rate.
- ✓ In general, nanotubes do not cause serious problem in ANS normal activities, however they are not completely biocompatible on the heart.

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