Cardiovascular effects of cetyl trimethyl ammonium bromide-protected gold nanoparticles

Water-dispersible monolayer-protected nanogold (MPNG), in which a layer of molecule covers the entire surface of the nanogold (gold nanoparticle), has been extensively used as a core for controlled drug release[1] and enhancement of radiotherapy.[2] Nanoparticles made up of polymer/surfactants[3] which contain, or are coated with, drugs are used for target-specific drug delivery. Since nanogold is a novel form of gold, only a few of its physical, chemical, and biological properties have been extensively studied in the past. Earlier studies on the treatment of cardiac and neurological disorders[4] with various gold salts and compounds prompted us to explore the cardiovascular effects of gold nanoparticles. The objective of the study was to find out the cardiovascular activity of gold nanoparticles, and its monolayer surfactant-protected derivatives, in the presence and absence of cardiovascular drugs such as atenolol and isoprenaline.

The cardiovascular action of cetyl trimethyl ammonium bromide (commonly known as CTAB, which is a cationic surfactant) monolayer-protected nanogold was studied. The nanogold solution was prepared by reducing gold ions in 100 ml of chloroauric acid solution (10^{-4} M), using sodium borohydride (0.01% w/v) as the reducing agent. This synthesis has been described by Brust *et al*.[5] To 90 ml nanogold solution (1.764 mg% of gold expressed in w/v), 10 ml of 10^{-3} M CTAB (aqueous) was added to obtain CTAB-protected nanogold (C-NG).

Four (two males and two females) healthy laboratory Wistar rats (300-400 g of body weight, aged 5-6 months) were used. The animals were procured from the National Toxicology Centre, Pune, and were well fed and housed in a clean environment at a temperature of 25 ± 1°C and a relative humidity of 45-55%, under a 12/12-hour light/dark cycle.

The animals were anesthetized using urethane i.p. (1.25 g/kg body weight). A Biopac 6 instrument (Biopac systems, Inc, USA) was used to record the ECG, heart rate, and blood pressure. The instrument electrodes were inserted in the rat in the formation of Lead II [Right arm (-ve)][Left arm (+ve)][Right leg (neutral)]. The blood pressure readings were obtained by cannulating the carotid artery. The C-NG, atenolol, and isoprenaline solutions were injected through a cannula inserted into the external jugular vein.

The volume of each dose of isoprenaline, atenolol, and C-NG did not exceed 1 ml/kg body weight of the animal. Isoprenaline was prepared using normal saline and 3 doses—100 nanogram, 300 nanogram, and 1 μg—were used. Atenolol in normal saline was administered at a dose of 1 mg/kg body weight. The heart rate, blood pressure, and ECG were recorded with each dose of a drug. Only when ECG and blood pressure returned to normal was the subsequent dose of the drug administered.

Separate control studies were conducted with nanogold and CTAB alone, and acute toxicity was also studied by administering nanogold and C-NG by oral/intravenous, intraperitoneal, and intramuscular routes for 8 days.

Before administration of C-NG the heat rate was 415 beats per min (bpm). It increased to 447 bpm after administration and then became normal, at 413 bpm, within 20-25 min, without any intervention [Table 1]. The ECG tracing of the rat before and after administration of C-NG (in the absence of isoprenaline and atenolol) are depicted in Figure 1A and B. We observed a decrease in the QRS complex and an increase in the time period between two repolarisation phases [Table 2].

Figure 2A shows the effect of C-NG on isoprenaline. The first dose of isoprenaline (100 nanogram, denoted by X) was given in the first minute and the other two doses in the fifth minute (300 nanogram, denoted by Y) and the seventh minute (1 μg, denoted by Z). The corresponding heart rates were 387 bpm, 400 bpm, and 413 bpm. The C-NG solution was added at the twelfth minute, when an initial sharp rise in blood pressure was observed (point A). After this three similar doses of isoprenaline were administered at intervals of 6 min, in response to which a larger sharp decrease in blood pressure was observed (denoted by B, C, and D) for each corresponding dose. The corresponding heart rates are 398 bpm (for B), 407 bpm (for C), and 417 bpm (for D). This shows that C-NG potentiates the action of isoprenaline [Table 3].

Figure 2B depicts the effect of C-NG on atenolol. The blood pressure showed a gradual decrease with atenolol (point A). The C-NG solution administered afterwards (point B) demonstrated a stabilizing effect on the blood pressure, preventing it from decreasing further. In other words, C-NG antagonized the effect of atenolol [Table 4].

The control studies with nanogold and CTAB did not affect the blood pressure in the presence or absence of isoprenaline or atenolol, but nanogold caused a mild bradycardia, the heart

Table 1

Effect of C-NG on heart rate

Heart rate with C-NG (beats per minute)	*Before C-NG*	*Immediately after C-NG*	*25 min after C-NG*
Rat no. 1	415	447	413
Rat no. 2	417	452	414
Rat no. 3	416	445	415
Rat no. 4	415	448	413
Mean	415.75	448	413.75
SD	0.829	2.549	1.658
Mean + SD	416.579	450.54	415.408
Mean - SD	414.929	445.451	412.092

Figure 1A: Normal ECG tracing (before administration of CTAB protected nanogold)

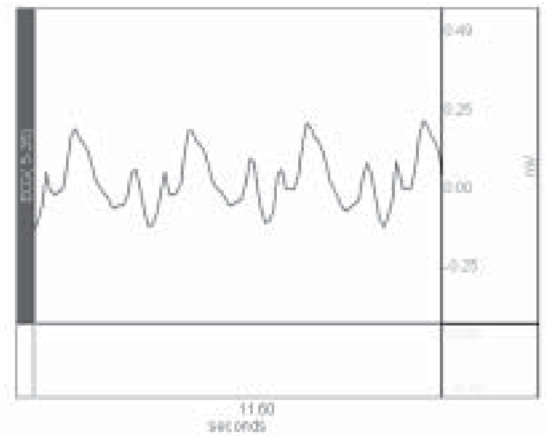


Figure 1B: ECG tracing (after administration of CTAB protected nanogold)

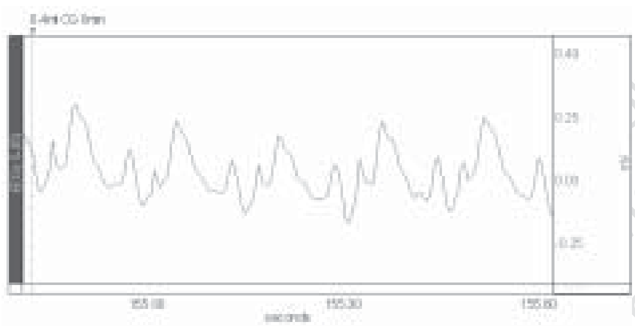


Table 2

Effect of C-NG on QRS complex and time between two repolarizations

ECG before C-NG (Time in seconds)	*QRS complex*	*Time between 2 repolarisation phases*
Rat No. 1	0.078	0.15
Rat No. 2	0.076	0.16
Rat No. 3	0.080	0.14
Rat No. 4	0.077	0.17
Mean	0.07775	0.155
SD	0.05	0.011
Mean + SD	0.1275	0.166
Mean - SD	0.0275	0.144
ECG after C-NG (Time in seconds)	*QRS complex*	*Time between 2 repolarisation phases*
Rat No. 1	0.072	0.168
Rat No. 2	0.071	0.170
Rat No. 3	0.074	0.169
Rat No. 4	0.073	0.171
Mean	0.0725	0.1695
SD	0.0011	0.0011
Mean + SD	0.0736	0.1706
Mean - SD	0.0714	0.11684
ECG 25 min after C-NG Administration (Time in seconds)	*QRS complex*	*Time between 2 repolarisation phases*
Rat No. 1	0.077	0.158
Rat No. 2	0.078	0.162
Rat No. 3	0.076	0.157
Rat No. 4	0.080	0.17
Mean	0.0775	0.16175
SD	0.0015	0.005
Mean + SD	0.0790	0.16675
Mean - SD	0.0760	0.15675

Figure 2A: Changes in blood pressure with isoprenaline (before and after administration of CTAB protected nanogold)

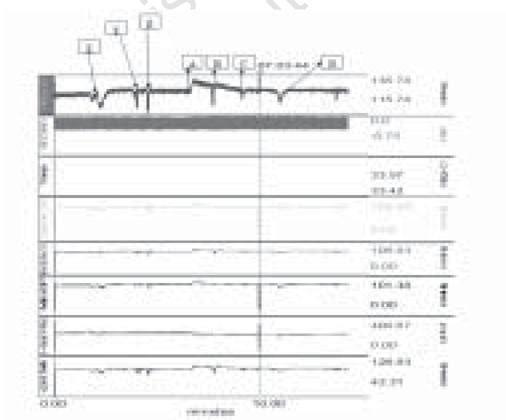
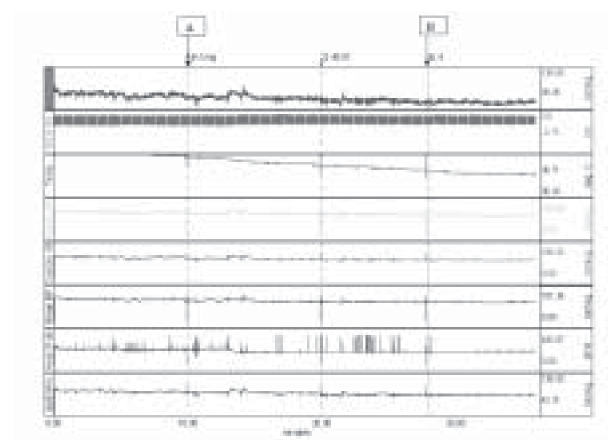


Figure 2B: Changes in blood pressure with atenolol (before and after administration of CTAB protected nanogold)



rate decreasing from 413 to 387 bpm. The toxicity study showed extremely mild kidney and liver necrosis.

C-NG enhanced the beta-adrenergic effect of isoprenaline and antagonized the beta-receptor blocking action of atenolol but did not alter the heart rate or blood pressure on its own. Though the drug appeared to be mildly toxic to the kidney and the liver in our study, Hainfield *et al.*[2] reported no toxic effect after 2 weeks of administration of nanogold in rats. He

Table 3

Effect of isoprenaline and C-NG on heart rate and blood pressure

Effect of isoprenaline on heart rate (beats per minute)	Before C-NG			After C-NG		
	100 ng	*300 ng*	*1 microgram*	*100 ng*	*300 ng*	*1 microgram*
Rat no. 1	387	400	413	398	407	417
Rat no. 2	390	402	415	397	410	420
Rat no. 3	392	404	414	400	409	418
Rat no. 4	386	401	412	399	408	419
Mean	388.75	401.75	413.5	398.5	408.5	418.5
SD	2.38	3.01	1.11	1.11	1.12	1.13
Mean + SD	391.1	404.76	414.61	399.61	409.62	419.63
Mean - SD	386.37	398.74	412.39	397.39	407.38	417.37
Effect of isoprenaline on (% decrease in blood pressure)	***100 ng***	***300 ng***	***1 microgram***	***100 ng***	***300 ng***	***1 microgram***
Initial Value in mm of Hg	102	101	99	104	101	103
Rat no. 1	-18.27	-24.37	-32.5	-20.4	-29.7	-39.8
Rat no. 2	-17.3	-22.55	-31.68	-21.5	-28.6	-40.4
Rat no. 3	-14.61	-22.81	-37.82	-19.8	-30.1	-41.3
Rat no. 4	-18.47	-24.07	-28.87	-21.6	-29.8	-39.4
Mean	-17.16	-23.45	-32.7175	-20.8	-29.5	-40.2
SD	-1.41	-1.35	-3.25	-0.75	-0.57	-0.71
Mean + SD	-18.57	-24.8	-33.96	-21.55	-30.07	-40.91
Mean - SD	-15.75	–22.1	–29.45	–20.05	–28.93	–39.49

Table 4

Effect of atenolol and C-NG on heart rate and blood pressure

Atenolol	*Heart rate (Initial Value=415 beats per minute)*			*Blood pressure (Initial Value=105 mm of Hg)*		
	Before C–NG	*After C–NG*	*Difference*	*Before C–NG (% decrease)*	*After C–NG (% decrease)*	*Difference (% decrease)*
Rat no. 1	385	401	16	–64.6	–12.7	–51.9
Rat no. 2	386	407	21	–63.7	–13.5	–50.2
Rat no. 3	388	409	21	–59.8	–12.8	–47
Rat no. 4	387	410	23	–62.6	–13.6	–49
Mean	386.5	406.5	20	–62.6	–13.15	–49.5
SD	1.65	3.57	2.59	–1.80	–0.4	–1.78
Mean + SD	388.15	410.07	22.59	–64.4	–13.55	–51.25
Mean - SD	384.85	402.9	17.41	–60.8	–12.75	–47.72

found that nanogold was cleared through the kidneys and the hematocrit values and enzymes remained within the normal ranges.

To the best of our knowledge, this is the first report on the cardiovascular activity of a novel nanomaterial, such as MPNG. The surface plasmon[6] of nanogold, which is responsible for its unique optical and electrical properties, as compared to gold in the bulk metal state, is also responsible for modifying the properties of the molecules attached to its surface. Sastry et al. have demonstrated the modification of the isoelectric point of amino acids[7] when capped onto nanogold. It is possible that this intrinsic property of nanogold could be the key reason for the cardiovascular activity which we have demonstrated with the present study.

Kishori Apte, Shantesh Hede*
Director of National Toxicology Center, Pune - 411 037;
*Division of Radiation Oncology and Hyperthermic Oncology and Medicine, Balabhai Nanavati Hospital, S.V. Road, Vile Parle (West) Mumbai - 400 056, India.
E-mail: hedeshantesh@hotmail.com

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