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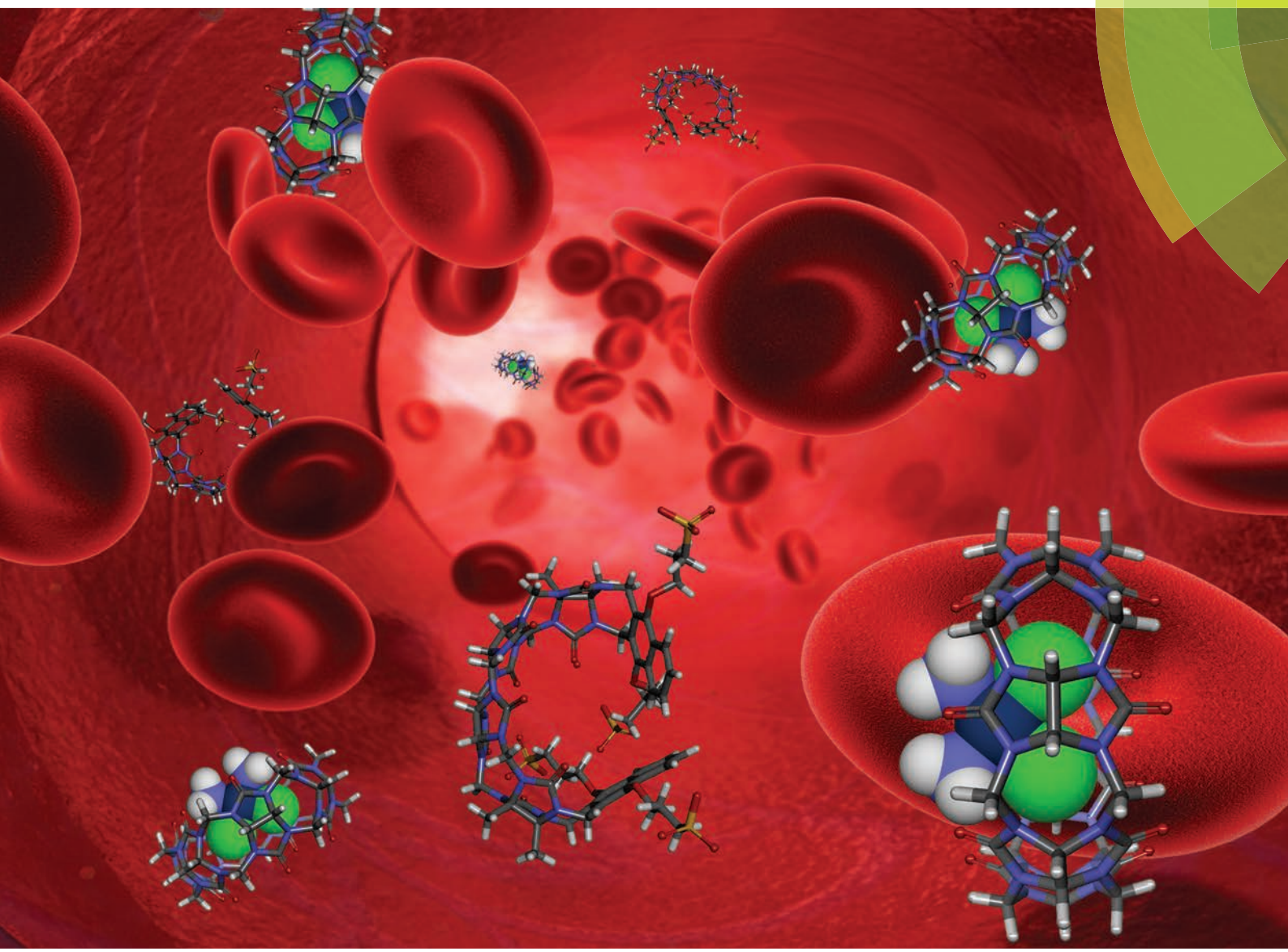

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The *ex vivo* neurotoxic, myotoxic and cardiotoxic activity of cucurbituril-based macrocyclic drug delivery vehicles

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The cucurbituril family of drug delivery vehicles have been examined for their tissue specific toxicity using *ex vivo* models. Cucurbit[6]uril (CB[6]), cucurbit[7]uril (CB[7]) and the linear cucurbituril-derivative Motor2 were examined for their neuro-, myo- and cardiotoxic activity and compared with β-cyclodextrin. The protective effect of drug encapsulation by CB[7] was also examined on the platinum-based anticancer drug cisplatin. The results show that none of the cucurbiturils have statistically measurable neurotoxicity as measured using mouse sciatic nerve compound action potential. Cucurbituril myotoxicity was measured by nerve-muscle force of contraction through chemical and electrical stimulation. Motor2 was found to display no myotoxicity, whereas both CB[6] and CB[7] showed myotoxic activity *via* a presynaptic effect. Finally, cardiotoxicity, which was measured by changes in the rate and force of right and left atria contraction, was observed for all three cucurbiturils. Free cisplatin displays neuro-, myo- and cardiotoxic activity, consistent with the side-effects seen in the clinic. Whilst CB[7] had no effect on the level of cisplatin's neurotoxic activity, drug encapsulation within the macrocycle had a marked reduction in both the drug's myo- and cardiotoxic activity. Overall the results are consistent with the relative lack of toxicity displayed by these macrocycles in whole animal acute systemic toxicity studies and indicate continued potential of cucurbiturils as drug delivery vehicles for the reduction of the side effects associated with platinum-based chemotherapy.

Introduction

Macrocycles are an important class of delivery vehicle that have the ability to form a range of host–guest complexes with drugs. This encapsulation can provide many benefits including: increased drug solubility, physical and chemical stability, taste masking, a reduction of drugs' toxic side effects and modulation of their cell uptake.[1] Furthermore, encapsulation can also provide controlled release ensuring that a high and consistent therapeutic concentration is available for a longer period of time compared to the free drug, thus increasing its bioavailability.

Although a number of macrocycles have already shown potential as delivery vehicles, there is still an increasing demand for a larger and more diverse selection as no one host is suitable for every drug. The cucurbit[*n*]urils (CB[*n*], where *n* represents the number of glycoluril units),[2,3] cyclodextrins,[4,5] and calixarenes[6,7] are the three main types of macrocycles that have been examined as drug delivery vehicles.

Cucurbit[*n*]urils (Fig. 1), are named for their resemblance to the shape of a pumpkin (cucurbitaceae). Native CB[*n*]s are synthesised from an acid-catalysed condensation reaction and are structurally made up of repeating glycoluril units linked by methylene bridges.[8] To date the CB[*n*] family consists of CB[5–8], CB[10] and CB[14].[8–10] All of the CB[*n*] homologues (except CB[14]) share a common height of 9.1 Å but differ in the diameter of their cavities. Cucurbit[14]uril does not have a normal cavity as a consequence of a twist in its structure, but instead adopts a figure-of-eight conformation.[10] Each native CB[*n*] (except CB[14]) contains two hydrophilic carbonyl portals and a hydrophobic interior and drug encapsulation is achieved *via* ion-dipole or hydrogen bonds to the CB[*n*] portals and through the hydrophobic effect upon CB[*n*]-drug host–guest complex formation.[11] Cucurbit[6]uril, CB[7] and CB[8] are most commonly used in drug delivery as the cavity of CB[5] is too small for drug incorporation and the cavity of CB[10] is too large for strong drug binding.[1]

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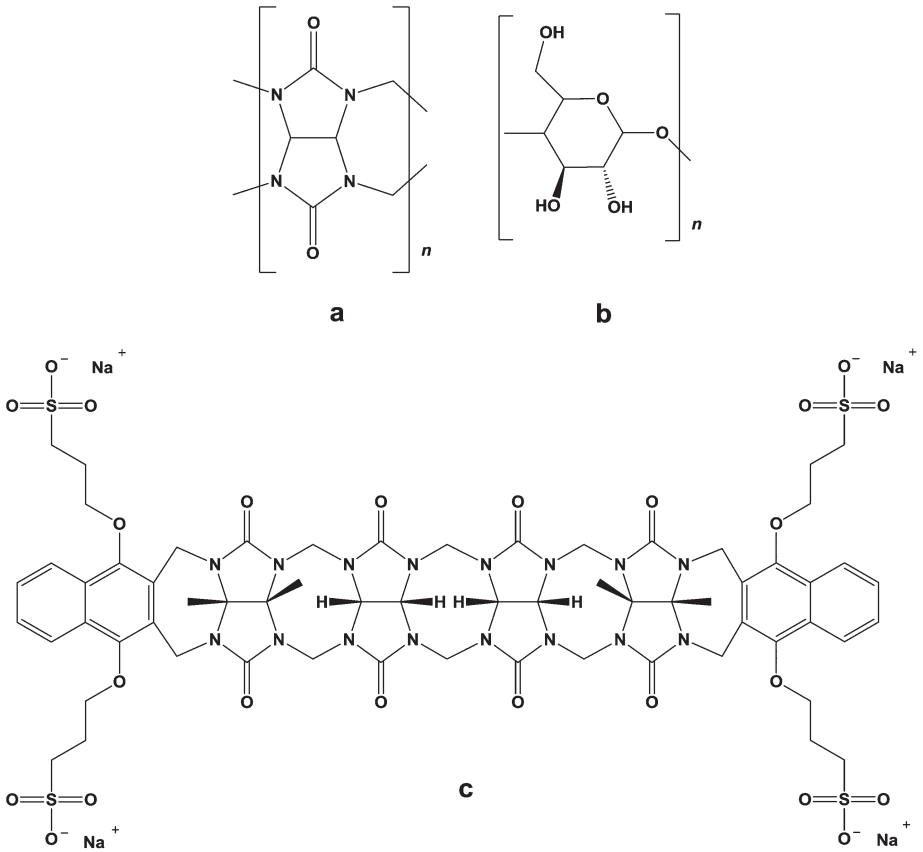

Fig. 1 The chemical structures of the macrocycles (a) cucurbit[*n*]uril, (b) *n*-cyclodextrin, and (c) Motor2.

Since the structural determination of CB[6] over three decades ago, several new cucurbituril derivatives have been developed, including Motor2 (Fig. 1). Whilst Motor2 is not a macrocycle but a linear chain, in solution it is able to fold to create a cavity similar to that of the native cucurbit[*n*]urils. The anionic charges of Motor2 means it is considerably more water soluble (18 mM in pure water) than the native cucurbiturils, and forms stronger bonds with cationic drugs through electrostatic interactions.[12]

The benefits of encapsulation by the CB[*n*]s are best demonstrated by the platinum-based drugs. In a recent study, it was shown that when encapsulated by CB[7], the anticancer drug cisplatin was able to overcome its own acquired resistance in an *in vivo* human tumour xenograft model *via* a pharmacokinetic effect.[13] Furthermore, encapsulation of the multinuclear platinum-based drug BBR3571 by CB[7] increased its maximum tolerated dose by 70% with the encapsulated complex being just as anticancer active as the free drug.[14] These results suggest a promising outlook for the use of CB[*n*] as drug delivery vehicles. Before CB[*n*]s can be approved for use in drug delivery; however, they must not only demonstrate that they are able to increase the efficacy of the encapsulated drugs and/or decrease their associated side effects but that they are also safe in terms of their systemic and organ specific toxicities.

Various *in vitro* and *in vivo* studies have thus far indicated that CB[*n*]s and their derivatives are both inert and non-toxic. Several studies have shown that at concentrations of up to 1 mM,[15,16] CB[7] displays no cytotoxic activity in a range of human and animal cell lines. When administered *in vivo* CB[7] has a maximum tolerated dose (MTD) of 250 mg kg^{-1}; intravenous injection of CB[*n*]s is limited more by their solubility rather than their side-effects. When a mixture of CB[6]–CB[8] was administered orally the MTD increased to 600 mg kg^{-1}.[17] Overall they show little sign of acute systemic toxicity. Motor2, has also been shown to be relatively non-toxic in human liver and kidney cells at concentrations up to 10 mM and when administered intravenously shows no systemic toxic effects at doses of up to 1230 mg kg^{-1}.[12]

In comparison, cyclodextrins, which are already approved for use in drug formulation, have been shown to be relatively non-toxic with lethal doses (LD_{50}) in rats and mice between 0.3 to 18.8 g kg^{-1} depending on the type of cyclodextrin and the route of administration.[18] β-Cyclodextrin, which is composed of seven glucopyranose units, is however known to be nephrotoxic when delivered intravenously.[18]

A limitation to these *in vitro* and *in vivo* systemic approaches is that little information can be gathered on the toxicity of cucurbiturils to specific organs and the mechanism by which they do so. Therefore, the use of *ex vivo* toxicological

models, in which the toxicity of the test compound is determined on intact whole tissue, can provide crucial and reliable predictions of the organ toxicity of CB[*n*]s in the human body.[19,20]

In this paper we report the use of *ex vivo* electrophysiological models to study the neurotoxic, myotoxic and cardiotoxic activity of native CB[*n*]s and Motor2 compared with β-cyclodextrin. In addition, the effect of CB[7] on the organ specific toxicity of cisplatin is examined.

Materials and methods

BALB/C mice and Sprague Dawley rats were obtained from Strathclyde University's in house breeding colonies that were originally sourced from Harlan, UK. Baby chicks were supplied from a breeding hatchery in Scotland (non-schedule 2 supplier). All animals were euthanized in compliance of the Code of Practice for the Humane Killing of Animals issued by the UK Home Office and in accordance with ethical guidelines of the Strathclyde Institute of Pharmacy and Biomedical Sciences. Cisplatin, β-cyclodextrin and CB[6] were bought from Sigma-Aldrich. Cucurbit[7]uril was bought from Dr Anthony Day, University of New South Wales, Australia. Motor2 was synthesised as previously described.[12]

Electrophysiological recordings from mouse sciatic nerve (neurotoxicity)

BALB/C mice weighing between 25–30 g were euthanised with CO_2. The sciatic nerve was carefully dissected from the knee to where it meets the spinal cord (3–5 cm in length) and immersed in HEPES-based physiological salt solution of the following composition: 150 mM NaCl, 5.4 mM KCl, 10 mM HEPES, 12 mM $NaHCO_3$, 0.4 mM KH_2PO_4, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 10 mM glucose, pH 7.4. The sciatic nerve was then de-sheathed under a stereo-microscope and laid across three inter-connecting chambers with ~70% of the nerve coiled in the middle chamber. The three chambers were filled with HEPES-based physiological salt solution to cover the nerve and electrical isolation between the three chambers was achieved with Vaseline. Electrical connections were made from the central chamber to one end chamber by means of Ag/AgCl electrodes. The remaining third chamber contained two platinum electrodes connected to a pulse generator. The middle chamber was connected to electrical grounds and the voltage from the third chamber was measured by a high input resistance amplifier. Electrical impulses were supplied to the nerve by a Grass S88 stimulator (0.4 Hz, 0.05 ms duration, 30 V) *via* a SIU 5A stimulus isolation unit (Grass Instrument Co. Quincy, MA, USA). Signals were amplified with a CED 1902 transducer (Cambridge Electronic Design, Cambridge, England), digitised with an analogue digital converter (DigiData 1200 Interface, Axon Instruments, Scotland), and analysed using Microsoft windows-based computer electrophysiological analysis software (WinWCP version 4.5.7; Dempster, 1988).

All experiments were carried out at room temperature and prior to the beginning of each experiment the sciatic nerve was incubated in HEPES-based physiological salt buffer for 30 min under constant super-maximal stimulation to demonstrate viability and consistency in the action potential recordings. If the action potential amplitude decreased by 10% the preparation was either remounted in fresh Vaseline or discarded. Compounds were added to the middle chamber (1 mM of CB[6], CB[7], Motor2, β-cyclodextrin, cisplatin and cisplatin@CB[7]). At the end of each experiment tetrodotoxin (TTX; 1 μg mL^{-1}) was added to the central chamber in order to determine the extent of de-sheathing. Sciatic nerves with adequate de-sheathing show a rapid decrease in the amplitude of their action potential when treated with TTX. If the sciatic nerve was not adequately de-sheathed the response to TTX is not as rapid and the results for these nerves were not used in the data analysis. Measurements are expressed as the mean ± SEM (n = 3).

Isolated chick biventer cervicis nerve muscle preparation (myotoxicity)

Chicks 3–15 days old were euthanised with CO_2. The back of their neck was plucked and the skin incised along the midline from the skull to below the base of the neck exposing both of the biventer cervicis muscle lying on either side of the midline. A thread was tied round the upper tendon muscle and a loop was tied at the bottom end of the neck muscle. The muscle was then cut away and kept in the following Krebs–Henseleit physiological salt buffer solution: 118 mM NaCl, 25 mM $NaHCO_3$, 11 mM glucose, 4.7 mM KCl, 1.2 mM $MgSO_4$, 0.3 mM KH_2PO_4, 2.5 mM $CaCl_2$ and equilibrated with a gas mixture of 95% O_2, 5% CO_2 to maintain a pH of 7.4. The loop was used to attach the preparation in the organ bath and ring electrodes were placed around the tendon to stimulate the muscle indirectly.

Prior to the addition of test compound, two control compound responses were obtained: 1 mM acetylcholine (ACh, 30 s) and 30 mM KCl (30 s). These control compounds were then washed by over flow with Krebs–Henseleit solution. After the KCl response was obtained, the preparations were allowed to equilibrate for 30 min before the addition of 300 μM of compound (CB[6], CB[7], Motor2, β-cyclodextrin, cisplatin and cisplatin@CB[7]). At the end of the experiment, the two control responses were repeated to determine the change in postsynaptic activity. Measurements are expressed as the mean ± SEM (n = 3–8).

Contractile recordings from rat atria (cardiotoxicity)

Male Sprague Dawley rats weighing between 250–350 g were killed by cervical dislocation and their heart was dissected and maintained in cold Krebs–Henseleit physiological salt solution and equilibrated with a gas mixture of 95% O_2/5% CO_2 to maintain the pH at 7.4. The left and right atria were carefully removed from the heart under a stereo-microscope and a small loop was tied at one end of both atria and a knot at the other end using a thin thread. The atria were then mounted into separate 10 mL organ baths containing Krebs–Henseleit

physiological salt solution, maintained at 37 °C and gassed with 95% O_2/5% CO_2 throughout the experiment. In the organ bath, one end of the atria was tied to a metal rod and the other end was attached, *via* a cotton thread, to a Grass force displacement transducer model FT03 connected to a powerlab/4SP analogue-to-digital recording system (AD Instruments) and displayed on a computer running Windows XP.

The right atria beats spontaneously due to the sinoatrial node, and a contraction was electrically evoked in the left atria by field stimulation (4 Hz, 2 ms duration, 10–15 V strength: Grass S88). A resting tension of 0.5 g was applied to both the left and right atria and maintained throughout the experiment. Prior to the start of each experiment, 10 μM noradrenaline (NA) was added to the organ bath, and tissue that did not respond was discarded. With the tissue that did respond, the NA was washed out and the atria allowed to equilibrate for 30 min before the addition of test compound (300 μM of CB[6], CB[7], Motor2, β-cyclodextrin, cisplatin and cisplatin@CB[7]). Measurements are expressed as the mean ± SEM (n = 3–5).

Results and discussion

The safe dose of cucurbiturils in animals is dependent on the size and type of the cucurbituril and its method of administration. The lowest maximum tolerated dose for CB[7] is 250 mg kg^{-1} when administered by injection. Assuming a model human size of 75 kg, then this equates to a concentration of approximately 3.2 mM. Therefore, in this study we have examined the toxicity of three different cucurbiturils (CB[6], CB[7] and Motor2) at a 1/3 and 1/10 of this maximum dose, giving 1 mM and 300 μM solution concentrations, respectively. To put the cucurbituril toxicity results into perspective they are compared with the known safe macrocycle, β-cyclodextrin, which has no reported neuro-, myo- or cardiotoxic side-effects.

Neurotoxicity

A desheathed mouse sciatic nerve preparation was chosen as a model to study the neurotoxicity of the macrocycles. The desheathing process involved the careful removal of the outer epineurium tissue that surrounds the nerve, thus allowing direct access to the nerve cells by the macrocycles.

The natural drop-off in nerve conduction was evaluated using a time control study that monitored the stability of the electrically generated nerve compound action potential (nCAP) over a period of two hours. Fig. 2 shows that within the first 40 min the sciatic nerve preparations are still 100% viable with no change in the amplitude of the nCAP. By 80 minutes the amplitude of the nCAP has decreased by only 5% ± 3.3 but by 120 minutes the sciatic nerve has lost 10% ± 4.2 of its viability. As a result, the toxicity experiments were carried using macrocycle solutions of 1 mM over a period of 80 minutes to ensure reliability of the results.

The results show that CB[7] and Motor2 induced a decrease in nCAP amplitude by 4% ± 0.2 and 13% ± 1.5, respectively,

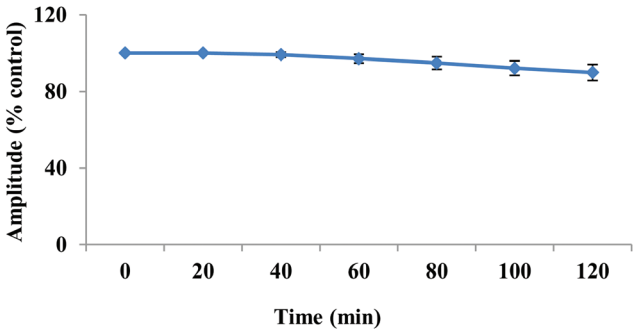

Fig. 2 The change in the amplitude of the nCAP of untreated sciatic nerves over a period of two hours. At 80 minutes, the nerves have only lost 5% of their normal conductance (n = 3).

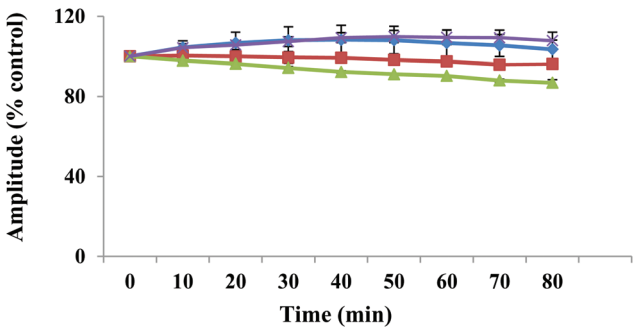

Fig. 3 The change in nCAP amplitude of the sciatic nerve after treatment with (blue) CB[6] (p = 0.3), (red) CB[7] (p = 0.7), (green) Motor2 (p = 0.1) and (purple) β-cyclodextrin (p = 0.1) after 80 minutes.

while CB[6] and β-cyclodextrin induced an increase in nCAP by 3% ± 4.6 and 7% ± 4.2, respectively (Fig. 3). β-Cyclodextrin is not known to be neurotoxic, so the similar increase in nCAP by CB[6] implies that the cucurbituril is probably not neurotoxic. The decrease in nCAP by CB[7] is the same magnitude as the natural drop-off in untreated samples indicating it is also not neurotoxic. Whilst Motor2 decreased nCAP by 13% there is no statistical difference (students paired t-test, p = 0.1) compared with untreated nerve. As such, all three cucurbiturils appear to display no neurotoxicity.

Effect of CB[7] on the neurotoxicity of cisplatin

Neurotoxicity is one of the major dose limiting side effects of cisplatin. Studies have shown that cisplatin primarily accumulates in the dorsal root ganglia (DRG) of neurons and predominantly induces sensory neuropathy characterised by severe loss of proprioception (body movement), sensation of pins and needles, and numbness of the feet.[21–23]

The effect of CB[7] on the neurotoxicity of cisplatin was investigated by analysing and comparing the effect of 1 mM cisplatin and cisplatin@CB[7] on the amplitude of nCAP.

The results show there is no statistical difference in the neurotoxicity of cisplatin compared with cisplatin@CB[7] (Fig. 4). At 80 min, cisplatin reduced the nCAP amplitude by 13% ± 4.7, which shows some neurotoxicity but not at the level

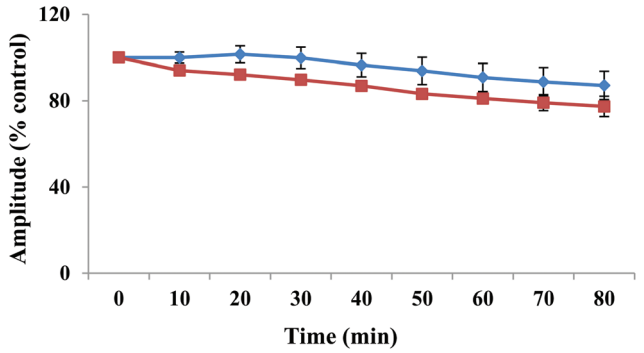

Fig. 4 The difference in the change of nCAP amplitude of the sciatic nerve after treatment with 1 mM of (blue) free cisplatin (p = 0.3) and (red) cisplatin@CB[7] (p = 0.1).

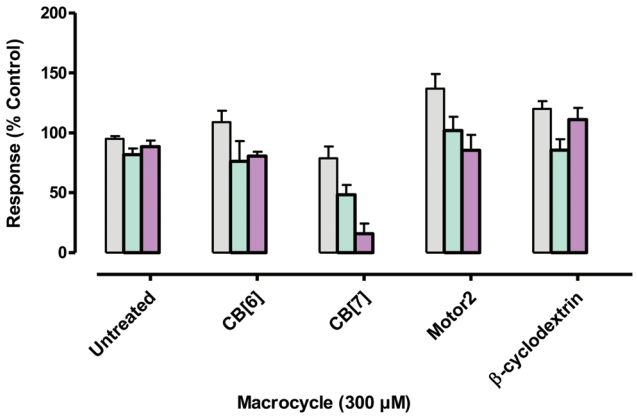

Fig. 5 The nerve-muscle's responses to (grey) ACh, (green) KCl and (purple) the electrically stimulated contraction at two hours after exposure to macrocycle for untreated nerves (n = 3), CB[6] (n = 3), CB[7] (n = 4), Motor 2 (n = 3) and β-cyclodextrin (n = 3).

normally observed in the clinic. The reason for the lower toxicity is most likely because the sciatic nerve is composed mainly of neuronal axons with smaller amounts of DRG, which are predominantly located in the spinal cord. Therefore, the low neurotoxicity caused by cisplatin in this experiment is due to the low number of DRG.

The results for cisplatin@CB[7] are similar to free cisplatin with a decrease in nCAP amplitude of 22.7% ± 6.6. The results therefore indicate that CB[7] does not have a neuro-protective effect for cisplatin.

Myotoxicity

The myotoxic activity of the macrocycles was examined using the isolated chick biventer cervicis nerve-muscle preparation. Under *ex vivo* conditions the muscle can be forced to contract using chemical or electrical stimulation. For chemical stimulation, the addition of exogenous acetylcholine (ACh) or KCl results in muscle contraction. The ACh acts by binding to nicotinic receptors located on the muscle membrane causing depolarisation followed by contraction (post-synaptic effect). Potassium chloride causes muscle membrane depolarisation resulting in calcium release into the synaptic cleft (the area between nerve and muscle). The calcium then binds to neuronal receptors which results in the release of ACh from the neuron ultimately causing muscle contraction (pre-synaptic effect).

Baseline results for the force of muscle contraction was determined using both electrical and chemical stimulation. The nerve-muscle was then exposed to the macrocycles and after two hours the force of muscle contraction was again determined (Fig. 5). The macrocycles are myotoxic if they demonstrate a statistically significant increase or reduction in the force of muscle contraction compared with baseline results. An increase in force of contraction due to exogenous ACh indicates that the compound tested may have anticholinesterase effect; cholinesterase is an enzyme located in the synaptic cleft that terminates signal transmission by breaking down acetylcholine activity therefore prolonging/increasing the effect of ACh. An increase in the lifetime of ACh will synergistically increase/prolong the response to KCl.

After two hours, the untreated nerve-muscle's response to ACh, KCl and its electrically stimulated contraction had all decreased by 4% ± 2, 18% ± 5 and 11% ± 5, respectively. Cucurbit[6]uril increased nerve-muscle response to ACh by 10% ± 10, and decreased its response to KCl and electrical stimulated contraction by 24% ± 17 and 20% ± 4, respectively. Cucurbit[7]uril decreased the nerve-muscle's response to ACh, KCl and the electrically stimulated contraction by 21% ± 10, 51.8% ± 8 and 84% ± 9, respectively. The cucurbituril-derivative, Motor2, increased nerve-muscle response to both ACh and KCl by (37% ± 12) and (2% ± 12), respectively, and decreased its electrically stimulated contraction by 15% ± 13. β-Cyclodextrin increased nerve-muscle response to ACh by 20% ± 7, decreased its response to KCl by 15% ± 9, and increased its electrical stimulated contraction by 11% ± 10.

Overall the largest myotoxic effect was observed after nerve-muscle exposure to CB[7]. Significant decreases in activity are apparent for KCl (p = 0.05) and electrical stimulation (p = 0.01), this indicates that CB[7] may be binding to and blocking the postsynaptic muscle's nicotinic receptors and therefore interfering with the depolarisation ability of the membrane. A small decrease in twitch *via* electrical stimulation is also observed after exposure to CB[6] (p = 0.3) although the decrease is considerably smaller in magnitude compared with CB[7]. Given that β-cyclodextrin also results in a decrease of electrically stimulated contraction (p = 0.9), and it is not a known myotoxic compound, the results may suggest that the electrical stimulation results for CB[6] and CB[7] are statistically decreased, however this may not result in *in vivo* toxicity.

Effect of CB[7] on the myotoxicity of cisplatin

The effect of CB[7] on the myotoxicity of cisplatin was investigated by analysing and comparing the effect of cisplatin and cisplatin@CB[7] on the response of the nerve-muscle's chemical and electrical stimulation.

The results show that cisplatin induced significant myotoxic activity when measured using both chemical and electri-

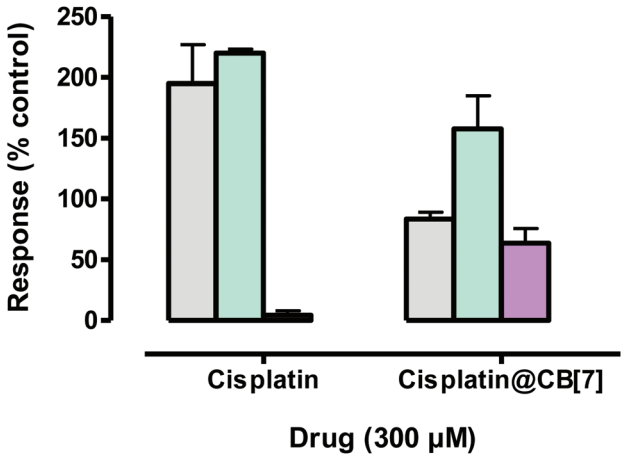

Fig. 6 The effect of cisplatin (n = 4) and cisplatin@CB[7] (n = 8) on the nerve-muscle's response to (grey) ACh, (green) KCl and (purple) the amplitude of its electrically stimulated contraction after two hours of exposure to macrocycle.

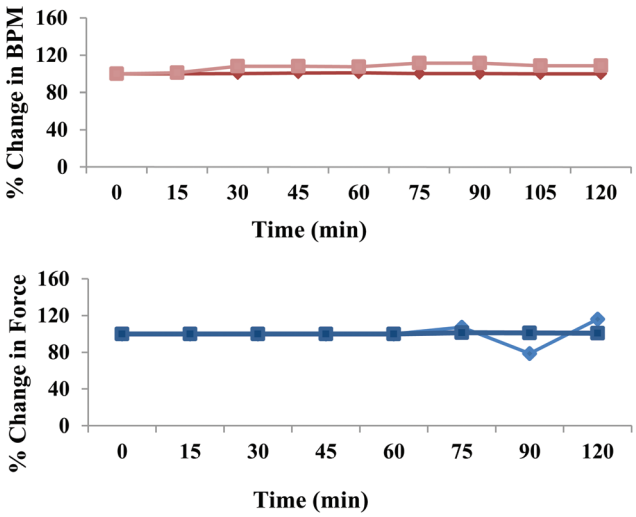

Fig. 7 The change in atria contraction rate (top panel) and force of contraction (bottom panel) in untreated right (dark red, dark blue) and left atria (light red, light blue) over a period of two hours.

cal stimulation (Fig. 6). Free cisplatin increased nerve-muscle contraction force to ACh and KCl by (96% ± 32, p = 0.005) and (121% ± 3, p = 0.001), respectively, and decreased the force of its electrically stimulated contraction by 96% ± 4 (p = 0.001) which is consistent with the peripheral neuropathy known to occur with cisplatin chemotherapy. The results also suggest that cisplatin may have anticholinesterase activity as demonstrated in previous studies and that this may also contribute in part to the neurotoxic side effects of cisplatin.[24] Encapsulation of cisplatin by CB[7] decreased nerve-muscle response to ACh by 17% ± 6 (p = 0.4), and increased its response to KCl by 59% ± 27 (p = 0.2). Cucurbit[7]uril showed a myo-protective effect as cisplatin@CB[7] decreased the electrical stimulated muscle contraction by only 36% ± 12 (p = 0.04) only; this is a reduction of the myotoxic activity of free cisplatin by 60%.

Cardiotoxicity

Heart atria from rats were used as a model to study the cardiotoxic activity of the macrocycles. The right atrium contains a natural pacemaker called the sinoatrial (SA) node which causes the atrium to contract naturally; however, such a pacemaker is absent in the left atrium and electrical stimulation is needed to initiate contraction.

Both atria were treated with each of the four macrocycles for a period of two hours. The function of the atria was studied by monitoring both the rate of atria contraction (BPM, beats per minute) and force of contraction (amplitude of contraction).

In the untreated atria (control), the results show that by the end of the experiment the left atrium was more stable than the right as no changes were observed in either its rate or force of contraction (Fig. 7). In contrast, after two hours the rate and force of right atrium contraction had increased by 8% ± 1 and 16% ± 1.9, respectively.

The cardiotoxicity results show that each cucurbituril portrayed significant cardiotoxic activity with Motor2 being the most cardiotoxic (Table 1). All of the macrocycles induced greater changes in the force of contraction compared with the contraction rate. Changes in the force of contraction are seen within the first 15–30 minutes after exposure to macrocycle, whereas a longer period of time (30–45 minutes) is required before changes in the rate of contraction are observed (Fig. 8).

Effect of CB[7] on the cardiotoxicity of cisplatin

Although uncommon, there are various clinical studies that have reported cardiotoxic effects of cisplatin. Toxicity occurs in

Table 1 A summary of the cardiotoxic changes induced by each macrocycle on the rate and force of both right and left atria contraction. The positive and negative signs indicate an increase or decrease in rate and force of contraction, respectively. The statistical significance of each result is given (p score based on student's paired t-test)

	Percent change in rate of contraction		Percent change in force of contraction	
Macrocycle (300 μM)	Right atrium	Left atrium	Right atrium	Left atrium
CB[6]	+12 ± 6 (p = 0.3)	+3.3 ± 1 (p = 0.3)	−41.7 ± 19.7 (p = 0.06)	−55 ± 6.1 (p = 0.06)
CB[7]	+31 ± 13.6 (p = 0.2)	−10 ± 3.5 (p = 0.4)	−29 ± 3.4 (p = 0.02)	−18 ± 6.8 (p = 0.3)
Motor2	+30 ± 19 (p = 0.4)	+15 ± 3.8 (p = 0.2)	−68 ± 3.1 (p = 0.001)	−60 ± 5.4 (p = 0.008)
β-Cyclodextrin	+32 ± 12 (p = 0.2)	+3 ± 3 (p = 0.5)	+1 ± 3.3 (p = 0.09)	−33 ± 4.1 (p = 0.04)

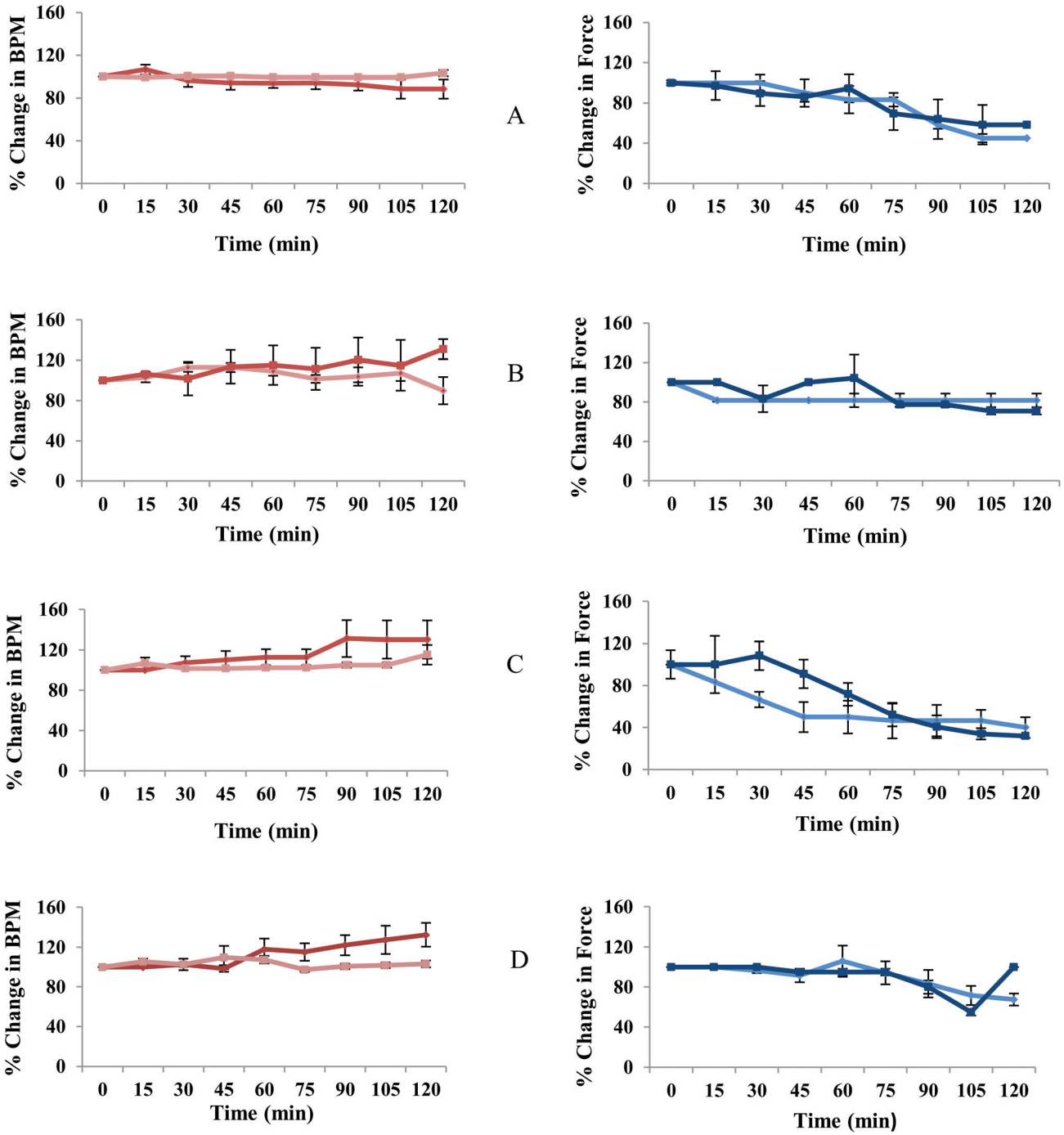

Fig. 8 The changes in the rate and force of contraction in both the right (dark red, dark blue) and left atria (light red, light blue) when treated with 300 μM of (a) CB[6], (b) CB[7], (c) Motor2 or (d) β-cyclodextrin over a period of two hours.

the form of atrial or ventricular arrhythmias, tachycardia, bradycardia and conduction abnormalities. Most of these cardiac problems are reported to be clinically silent and occurring within hours of drug infusion.[25–27]

The protective effect of CB[7] on the cardiotoxic activity of cisplatin was investigated using free cisplatin and cisplatin@CB[7] in the unpaced right atrium. The right atrium was chosen for this study as cisplatin exhibited no cardiotoxic activity in the left paced atrium (data not shown).

The results show that cisplatin induced cardiotoxic activity by reducing both the rate and force of contraction by 68.8% ± 8.4 (p = 0.07) and 53.7% ± 17.0 (p = 0.2), respectively (Fig. 9). Furthermore, cisplatin induced a gradual and dramatic increase in the atria's force of contraction by 250% within the first 60 min, after which it was reduced by 54% by the end of the experiment. When encapsulated by CB[7], the cardiotoxic activity of cisplatin was significantly reduced; cisplatin@CB[7] induced only an 11% ± 5.6 (p = 0.2) decrease in the rate of

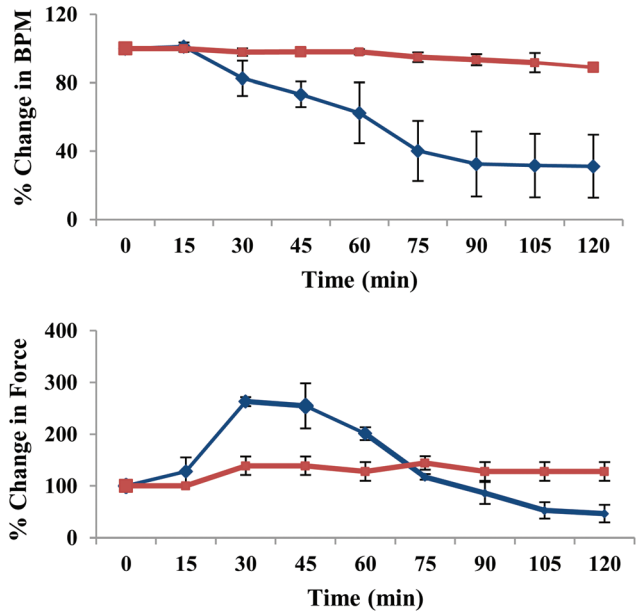

Fig. 9 The effects of (blue) cisplatin and (red) cisplatin@CB[7] on right atria contraction rate (top panel) and force of contraction (bottom panel) over a period of two hours.

atria contraction, and increased the force of contraction by only 27% ± 18.0 (p = 0.2). As well as reducing the magnitude of change in rate of contraction, CB[7] also stabilised the force of contraction compared to free cisplatin. After 60 min the force of contraction increased by just 27% ± 18.0 and was maintained at this magnitude throughout the experiment.

Conclusion

In this study, the cucurbituril macrocycles CB[6] and CB[7] and their derivative, Motor2, were tested for their neuro-, myo- and cardiotoxic activity and the results compared with the known safe β-cyclodextrin using *ex vivo* models.

The results show that all of the macrocycles showed no neurotoxic activity within the time frame tested as there was no statistically measurable difference between the changes in nCAP induced by the cucurbiturils compared to that caused by β-cyclodextrin or control. In the myotoxicity study, CB[7] showed the greatest myotoxic activity by causing the greatest reduction in the electrically stimulated nerve-muscle contraction, whilst Motor2 and β-cyclodextrin displayed the least myotoxic activity. All of the macrocycles showed cardiotoxic activity by predominantly reducing the force of atria contraction compared to changes in the rate of its contraction, with Motor2 being the most cardiotoxic.

The effect of drug encapsulation by CB[7] on these specific tissue toxicities was tested using the platinum-based anticancer drug cisplatin. While free cisplatin displayed toxicity to all three tissue types, when encapsulated by CB[7], the extent of its myo- and cardiotoxic activity were significantly reduced while no statistical change in toxicity was seen in the neurotoxicity studies. These *ex vivo* results are consistent with the lack of acute systemic toxicity seen by for CB[*n*]s when previously tested for their *in vitro* and *in vivo* systemic toxicities and also show the advantages of using CB[7] as a drug delivery vehicle in providing protection from some of the side effects associated with the platinum-based drugs.

Overall, whilst some toxicity of the macrocycles was observed for myo- and cardio toxicity, these experiments represent very high doses with direct access to the tissues. In a human clinical setting, the dose used will be 2- to 10-fold lower. For example, if used as a protective agent for cisplatin then the actual dose of CB[7] will be 80 to 160 μM (assuming a cisplatin dose of 60 to 120 mg m^{-2}).[28] From this, much of the dose will be quickly cleared *via* the kidneys and the dose to which the nerves, muscle and heart will be exposed will be significantly lower still. The results, however, do warrant further investigation into the toxicity of CB[*n*]s to establish their safety.

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