

Electronic Supplementary Material

Safety evaluation of nanodiamond-doxorubicin complexes in a Naïve Beagle canine model using hematologic, histological, and urine analysis

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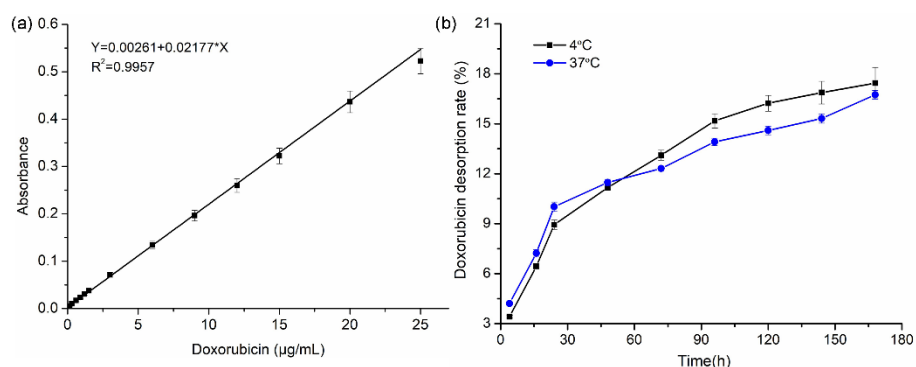


Figure S1 The loading efficacy and stability of NDX complex (a) DOX standard curve (b) the drug release in aqueous solution for 168 hours at 4 °C and 37 °C.

Table S1 Clinical Observation

No.	Observation Finding	Observation Time Point
Group 1-DOX (IV, Male)		
101	Head pruritus	Postdose 0, 7 min
	Skin discolored (red)	Postdose 0, 7, 14, 30, 60, 90, 120 min
	Activity decreased	Postdose 14 min, ~77 h, ~96 h
	Emesis	Postdose 14 min, ~53 h, ~60 h, ~72 h, ~96h
	Feces soft	Postdose ~72 h, ~96 h
	Feces discolored (red)	Postdose ~72 h, ~96 h
102	Skin discolored (red)	Postdose 0, 7, 16, 30 min
	Head pruritus	Postdose 0, 7, 16 min
	Emesis	Postdose ~48 h, 53 h, 60 h, ~72 h
	Feces soft	Postdose ~72 h
	Feces discolored (red)	Postdose ~72 h
	Prostrate	Postdose ~72 h, ~77 h
	Found dead	Postdose ~81 h
Group 2-ND (IV, Male)		
201	Feces	Postdose 5 min
	Emesis (white bubbles, 7-8 mL)	Postdose 5 min
	Limbs weakness	Postdose 8, 13, 16, 24 min
	Righting reflex impaired	Postdose 13, 16 min
	Prostrate	Postdose 24 min
	Emesis (mild yellow liquid, ~2 mL)	Postdose 68 min
202	Head movements – jerky	Postdose 2 min
	Scratching excessive	Postdose 2, 4, 8 min
	Skin discolored (red, periocular, perioral)	Postdose 4, 8, 15, 18, 29 min
	Activity decreased	Postdose 8, 15, 18, 29, 61 min
	Emesis (yellow mucoid, ~15 mL)	Postdose 15 min
	Skin thickened and leathery	Postdose 18, 29, 61 min
Group 3-NDX (IV, Male)		
301	Head movements – jerky	Postdose 1, 8, 11, 15, 30 min
	Scratching excessive	Postdose 1, 8, 11, 15 min
	Activity decreased	Postdose 1, 8, 11, 15, 30 min
	Skin thickened and leathery	Postdose 11, 15, 30, 48 min
302	Head movements – jerky	Postdose 1 min
	Skin discolored (red, periocular, periotic, perioral)	Postdose 2, 7, 14, 29, 35, 58 min
	Scratching excessive	Postdose 2, 7, 14, 29 min
	Skin thickened and leathery	Postdose 7, 14, 29, 35, 58 min
	Activity decreased	Postdose 14, 29, 35 min
303	Head movements – jerky	Postdose 1, 4, 8 min
	Scratching excessive	Postdose 1, 4, 8, 15 min
	Activity decreased	Postdose 4, 8, 15, 30, 60 min
Group 4-NDX (IV, Female)		
401	Head movements – jerky	Postdose 1, 4 min
	Scratching excessive	Postdose 1, 4, 8 min
	Activity decreased	Postdose 4, 8, 15, 30, 60 min
402	Abnormal gait	Postdose 2, 7 min
	Scratching excessive	Postdose 2, 7, 15 min

Table S1 Clinical Observation (<i>continued</i>)	
Head movements – jerky	Postdose 2 min
Salivation	Postdose 2, 7, 15 min
Skin discolored (red, periocular, periotic, perioral)	Postdose 2, 7, 15, 29, 60 min
403 Scratching excessive	Postdose 2, 7, 15 min
Head movements – jerky	Postdose 2 min
Skin discolored (red, periotic, perioral)	Postdose 2, 7, 15, 29 min
Group 5-NDX (HPV, Male)	
501 Feces	Postdose 3 min
Activity decreased	Postdose 3, 6, 12, 15, 16, 20 min
Breathing rapid	Postdose 6, 12, 15, 16, 20, 30, 60 min
Abnormal gait	Postdose 15, 16 min
Urination	Postdose 16 min
Righting reflex impaired	Postdose 20, 30, 60 min
Prostrate	Postdose 30, 60 min
502 Activity decreased	Postdose 1, 5, 15, 29, 60 min
503 Activity decreased	Postdose 2, 3, 7, 9, 16, 32, 60 min
Emesis (white liquid, ~15 mL)	Postdose 3 min
Prostrate	Postdose 9, 16 min

Table S2 Canine and drug administration information in this study

Group No.	Animal No.	Animal ID	Sex	Body Weight (kg)	Drug Type	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Dose Route
1	101	8694746	Male	13.09	DOX	3.648	1	3.648	IV
1	102	8706370	Male	14.88	DOX	3.648	1	3.648	IV
2	201	8581089	Male	13.09	ND	3.648	1	3.648	IV
2	202	8490687	Male	14.88	ND	3.648	1	3.648	IV
3	301	8544931	Male	9.25	NDX	3.648	1	3.648	IV
3	302	8551376	Male	10.92	NDX	3.648	1	3.648	IV
3	303	8570362	Male	12.2	NDX	3.648	1	3.648	IV
4	401	8610933	Female	11.21	NDX	3.648	1	3.648	IV
4	402	8496863	Female	10.49	NDX	3.648	1	3.648	IV
4	403	8487953	Female	10.09	NDX	3.648	1	3.648	IV
5	501	8573982	Male	11.35	NDX	3.648	1	3.648	HPV
5	502	8619362	Male	10.21	NDX	3.648	1	3.648	HPV
5	503	8585262	Male	10.61	NDX	3.648	1	3.648	HPV

Note: The unmodified DOX, ND, and DOX equivalent in synthetic NDX were all 1 mg/mL.

Table S3. Mass spectrum parameters of DOX, NDX and IS

Drug name	Q1	Q3	Dwell time	DP (v)	EP (v)	CE (v)	CXP (v)
DOX	544.1	320.9	100	45	10	40	10
NDX	544.1	320.9	100	100	10	40	10
Tolbutamide (IS)	271.1	172.0	100	70	10	18	10