Biochim Biophys Acta 1994; 1191: 384 – 8.
11 Bouchelouche P, Friche E, Sehested M, Jensen PB, Skovsgaard T Cytosolic free Ca^{2+} in daunorubicin and vincristine resistant Ehrlich ascites tumor cells. Drug accumulation is independent of intracellular Ca^{2+} changes.
Biochem Pharmacol 1991; 41: 243 – 53

2-苯-3-(3′,5′-双吗啉甲基-4′-羟基)-苯甲酰吲哚(HWL-12)逆转肿瘤多药抗药性

符立梧[1], 潘启超, 黄文龙[2], 杨小平
([1] 中山医科大学肿瘤研究所, 广州 510060, 中国; [2] 中国药科大学药化研究室, 南京 210009, 中国)

关键词 多种抗药性; 吲哚类; HWL-12; 阿霉素; Fura-2; 钙; 培养的肿瘤细胞; 维拉帕米; 依他酸

目的: 探讨吲哚衍生物 HWL-12 逆转 MDR 的作用及其作用机制. **方法**: 细胞毒的测定用 MTT 法; P-糖蛋白的测定用我们新近建立的 Fura 2-AM 法. 细胞内阿霉素(Dox)的积累测定用荧光分光光度计法. **结果**: HWL-12 能显著地增加 MDR 细胞内 Fura-2 的积累和逆转 MDR 17.2 倍. 在 HWL-12 的作用下, MDR 细胞内阿霉素的积累亦明显增加, 但在介质中高钙或低钙离子浓度对 Dox 积累均无影响. **结论**: HWL-12 具有较强的逆转作用, 它的逆转机制可能与增加 MDR 细胞内阿霉素的积累有关, 而与钙离子浓度无关.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1998 Jan; 19 (1): 80 – 84

Effect of catecholamic acid on detoxication and distribution of $NiCl_2$ in mice and rats[1]

YAN Xue-Ming[2], TAO Zheng-Qin, LIANG You-Yi, CHEN Zhen-Jia, ZHANG Jian-Shi, XU Xin-Hua
(*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China*)

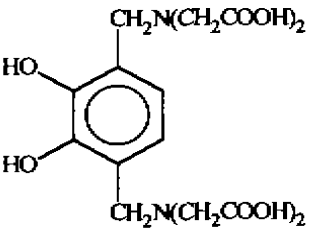
KEY WORDS chelating agents; catecholamic acid; nickel; lethal dose 50; autoradiography; tissue distribution

AIM: To study the effect of catecholamic acid (CBMIDA) on detoxication of $NiCl_2$. **METHODS**: Mice and rats were injected sc or im CBMIDA immediately after ip $NiCl_2$. Each mouse was injected ip CBMIDA after iv $^{63}NiCl_2$ 185 kBq, and radioactivities of various tissues were measured with liquid scintillation counter at 24 h. The localization of ^{63}Ni was shown by the whole-body autoradiography. **RESULTS**: CBMIDA sc 0.5 – 1.5 $g \cdot kg^{-1}$ markedly reduced the mortality from acute poisoning of ip $NiCl_2$ 500 $mg \cdot kg^{-1}$. After ip $NiCl_2$ in mice, the LD_{50} was 82.7 $mg \cdot kg^{-1}$. Mice were injected sc CBMIDA 1.5 or 2.5 $g \cdot kg^{-1}$ after Ni poisoning, the LD_{50} of $NiCl_2$ were raised to 789 or 820 $mg \cdot kg^{-1}$, respectively. The LD_{50} of $NiCl_2$ was 39 $mg \cdot kg^{-1}$ in rat. If CBMIDA was injected im 0.5 $g \cdot kg^{-1}$ after ip $NiCl_2$, the LD_{50} was 332 $mg \cdot kg^{-1}$. CBMIDA 1.5 $g \cdot kg^{-1}$ im after iv $^{63}NiCl_2$, decreased the contents of ^{63}Ni in blood and lung of mice *vs* control mice at 24 h. The contents of ^{63}Ni in brain, heart, spleen, and kidney were similar to those of the control mice. The content of ^{63}Ni in bone was more than the control. The excretions of ^{63}Ni through urine and feces were not increased by CBMIDA at 24 h. The whole-body autoradiograpy showed that the radioactivity was highly localized in the kidney, lung, and Harder's gland. There was a moderate level of ^{63}Ni in the liver, bone, skin, and blood. A pronounced accumulation occurred in the bone. There was a marked reduction of ^{63}Ni in the lung, skin, liver, and blood after ip CBMIDA. **CONCLUSION**: The CBMIDA markedly raised the survival rate of nickel-poisoned mice and rats, and decreased ^{63}Ni levels in lung and blood.

[1] Project supported by the National Natural Science Foundation of China. No 3870510.
[2] Phn 86-21-6431-1833, ext 404 Fax 86-21-6437-0269.
Received 1996-12-19 Accepted 1997-09-15

Nickel compounds widely used in industry were absorbed into the body and deposited mainly in lung, so the lungs are the critical organ after exposure to nickel. A long period of exposure to nickel compounds may cause lung cancer[1], and most chelating agents are ineffective to treat nickel poisoning.

Catecholamic acid [catechol-3,6-bis(methyleneiminodiacetic acid), CBMIDA] first synthesized by Prof XIE Yu-Yuan in our Institute was a chelating agent of a low toxicity[2], and increased excretion of uranium[3] and plutonium[4] in rats.



Catecholamic acid (CBMIDA)

The purpose of the present study was to explore the effects of CBMIDA on detoxication, removal, and tissue distribution of $NiCl_2$.

MATERIALS

Kunming mice (♀) and Sprague-Dawley rats (♀) were obtained from Shanghai Experimental Animal Center, Chinese Academy of Sciences, Certificate № 005.

Catecholamic acid was synthesized by Department of Synthetic Chemistry in our Institute, purity >95 % and mp 184 ℃. It was dissolved in distilled water with the aid of $NaHCO_3$, pH 6 – 7, and prepared before 30 min administration. $NiCl_2$(AR) was produced by Shanghai Qingong Chemical Plant. $^{63}NiCl_2$ was made by the Radiochemical Center Ltd, UK (radioactivity 247 GBq · L^{-1}). The X-ray film was LKB Ultrafilm S-161 25 Bromma, Sweden. The whole-body section was made on Lipshaw Cryotome 1800-N freezing microtome, USA. Liquid scintillation counter (model YSJ-78) was made by Shanghai Institute of Nuclear Research, Chinese Academy of Sciences.

METHODS AND RESULTS

Detoxication of nickel by CBMIDA Mice (weighing 20.0 ± s 1.6 g) injected ip $NiCl_2$ 500 mg·kg^{-1} were divided into 5 groups, 20 mice/group. Then the control group mice were injected sc 0.9 % saline, and other 4 groups were immediately injected sc CBMIDA 0.5, 1, 1.5, and 2 g·kg^{-1}, respectively. The mortality was recorded within 3 d. The diarrhoea, respiratory distress, and lethargy were noted in toxicity tests. All mice of control group died. In the CBMIDA-treated groups, 0.5 – 1.5 g·kg^{-1}, exhibited a dose-response relationship (Tab 1).

Tab 1. Detoxificating effect of sc catecholamic acid (CBMIDA) after ip $NiCl_2$ 500 mg·kg^{-1} within 3 d. $^cP<0.01$ *vs* 0; $^dP>0.05$, $^eP<0.05$ *vs* 0.5; $^fP>0.05$ *vs* 1.

CBMIDA/g·kg^{-1}	Injected mice	Survived mice
0	20	0
0.5	20	13[c]
1.0	20	17[cd]
1.5	20	20[ef]
2.0	20	20[ef]

Various chelating agents on detoxication of nickel Mice (weighing 19.8 ± s 1.3 g, divided into 9 groups, 40 mice/group) were injected ip $NiCl_2$ 70, 150, 250, and 500 mg·kg^{-1}, (n = 10). After nickel poisoning, 7 chelating agents were injected sc: CBMIDA 1.5 or 2.5; sodium dimercaptosuccinate (Na-DMS) 1; qunamic acid (QA) 0.5; penicillamine (PCA) 0.3; 1,2-cyclohexylene dinitrilotetraacetic acid (CDTA) 0.15; edetic acid 0.15; dithiocarb (DDC) 0.5 g·kg^{-1}. CBMIDA remarkably raised the survival rate of mice, as compared with other chelating agents. The effect of CBMIDA 1.5 g·kg^{-1} was similar to that of 2.5 g·kg^{-1} (Tab 2).

Tab 2. Survived mice after sc 7 chelating agents after ip $NiCl_2$ (n = 10).

Chelator/g·kg^{-1}		$NiCl_2$/mg·kg^{-1} 70	140	250	500
		3	0	0	0
CBMIDA	1.5	10	10	10	10
	2.5	10	10	10	9
CDTA*	0.15	10	1	2	0
Dithiocarb	0.5	0	1	1	0
Edetic acid	0.15	10	8	2	0
Na-DMS	1	10	10	6	2
Penicillamine	0.3	10	10	0	0
Qunamic acid	0.5	10	7	0	0

*1,2-cyclohexylene dinitrilotetraacetic acid

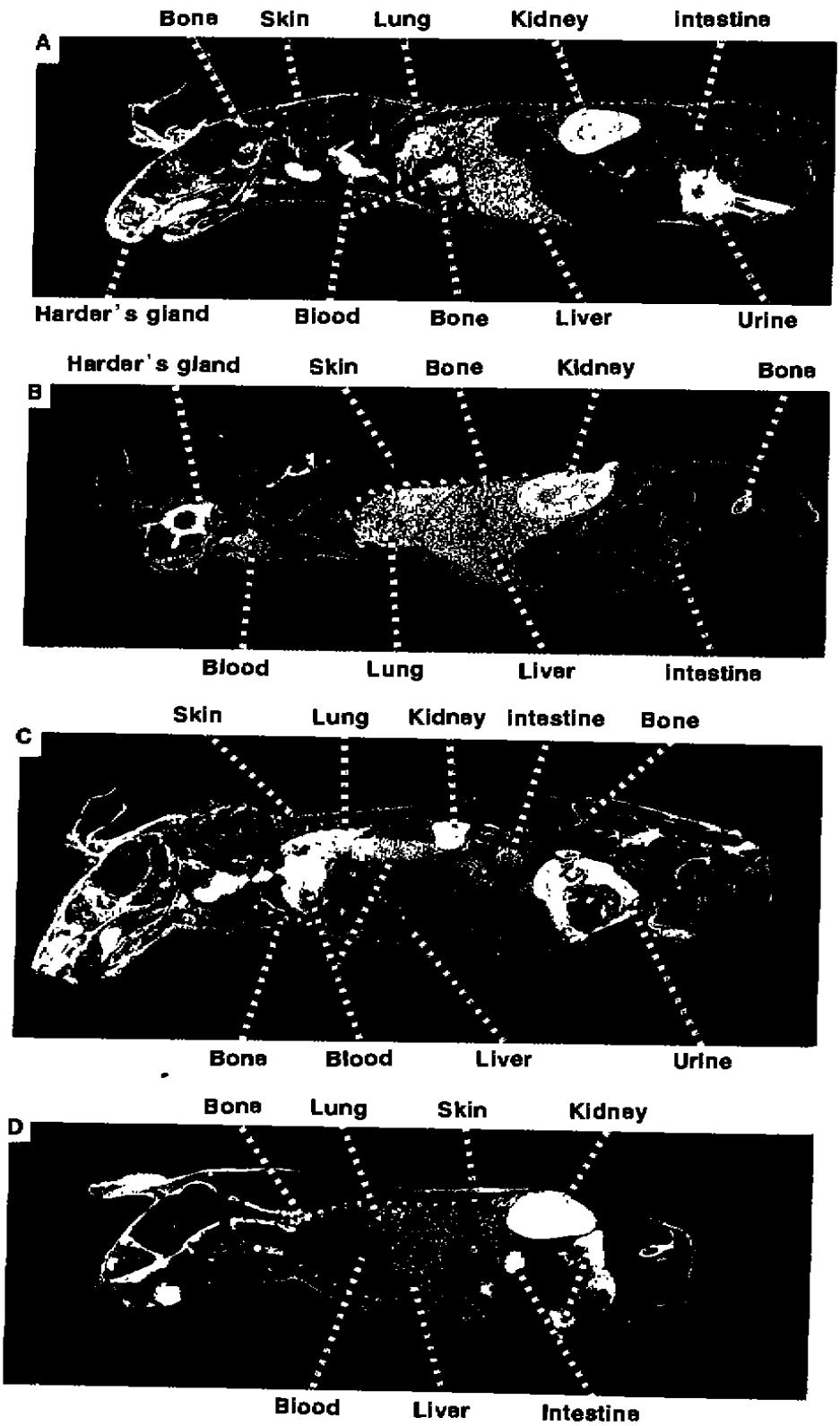


Fig 1. Autoradiograms from ♀ mice iv $^{63}NiCl_2$ alone or followed by CBMIDA 3 h after Ni (A) or Ni + CBMIDA (B); 8 h after Ni (C) or Ni + CBMIDA (D).

Effects of chelating agents on LD_{50} of $NiCl_2$ Mice (n = 260, weighing 20.4 ± s 1.5 g) were divided into 5 groups. Control group was injected ip only $NiCl_2$ at various doses (n = 10). Other 4 groups were respectively injected sc: CBMIDA 1.5 or 2.5, Na-DMS 1, edetic acid 0.15 g·kg^{-1} after ip $NiCl_2$. The 3-d LD_{50} (95 % confidence limits) were calculated by Finney's way.

After ip a single dose to mice, the LD_{50} of nickel chloride was 82.7 mg·kg^{-1}, they were immediately injected sc chelating agents, the LD_{50} of $NiCl_2$ was raised as the following order: CBMIDA > Na-DMS > edetic acid (Tab 3).

Tab 3. LD_{50} of ip $NiCl_2$ alone or followed by chelating agents.

Species	n	Chelator	Dose/ g·kg^{-1}	Route	LD_{50}/mg·kg^{-1} (95 % confidence limits)
Mice	70	–	–	–	82.7 (76.9–88.9)
	50	CBMIDA	1.5	sc	789 (693–899)
	60		2.5	sc	820 (693–970)
	40	Edetic acid	0.15	sc	187 (152–230)
	40	Na-DMS	1	sc	310 (245–392)
Rats	50	–	–	–	38.8 (36.7–41.0)
	50	CBMIDA	0.5	im	332 (305–362)

Rats (n = 100, weighing 182 ± s 21 g) were divided into 2 groups. The toxic group was injected ip various doses of $NiCl_2$. The detoxification group was injected im CBMIDA 0.5 g·kg^{-1} after ip $NiCl_2$. The LD_{50} of $NiCl_2$ was elevated from 39 to 332 mg·kg^{-1}, about 8.6 times of that in toxic group (Tab 3).

Effect of CBMIDA on ^{63}Ni tissue distribution and elimination Mice (weighing 20.4 ± s 0.5 g) were divided into 2 groups, 10 mice/group. The control group was injected iv $^{63}NiCl_2$ 185 kBq/mouse; the treated group was injected ip CBMIDA 1.5 g·kg^{-1} after iv $^{63}NiCl_2$. Each mouse was kept in a metabolism cage for 24 h, and then killed. The heart, lung, brain, liver, spleen, kidney, bone, and blood were weighed, and 7 – 15 mg, were digested in 0.1 mL formic acid and 0.1 mL H_2O_2 at 80 ℃ for 30 min. Radioactivity was measured in 8 mL of 0.01 % POPOP and 0.4 % PPO with liquid scintillation counter for 1 min. In the CBMIDA-treated group, the contents of ^{63}Ni were reduced remarkably in blood and lung, but increased in bone. ^{63}Ni in urine, feces, and other tissues were similar to those of control group at 24 h (Tab 4).

Tab 4. Tissue radioactivity at 24 h after iv $^{63}NiCl_2$ 185 kBq and ip CBMIDA 1.5 g·kg^{-1} in mice n = 10. $^aP > 0.05$, $^cP < 0.01$ *vs* control.

	Radioactivity (kBq/g wet tissue) Control	CBMIDA
Blood (L)	967 ± 367	433 ± 100^c
Bone	0.52 ± 0.13	1.8 ± 0.7^c
Brain	0.55 ± 0.22	0.38 ± 0.18^a
Heart	0.60 ± 0.25	0.47 ± 0.22^a
Kidney	2.9 ± 1.1	2.8 ± 1.2^a
Liver	0.8 ± 0.4	0.7 ± 0.3^a
Lung	1.9 ± 0.5	0.55 ± 0.22^c
Spleen	1.1 ± 0.6	1.1 ± 0.8^a
Urine (24 h)	105 ± 33	109 ± 37^a
Feces (24 h)	15 ± 14	17 ± 16^a

Effect of CBMIDA on ^{63}Ni localization in mice Mice (n = 4, weighing 28 g) injected iv $^{63}NiCl_2$ 370 kBq/mouse were divided into 2 groups, 2 mice served as control, the other 2 mice were immediately injected ip CBMIDA 1.5 g·kg^{-1}. At 3 and 8 h, respectively, 1 mouse in each group was anesthetized with ether, and frozen in mixture of dry ice and hexane (−75 ℃). Autoradiography was made[5]. Whole-body sections were made 20 μm in thickness, at −20 ℃, the X-film was exposed for 43 wk for the 3-h group, and exposed for 48 wk for the 8-h group.

At 3 h after the mice were injected iv $^{63}NiCl_2$, the concentrations were high in kidney, lung, and Harder's gland; moderate in bone, liver, skin, and blood. There were trace radioactivities in intestines (Fig 1A). In the CBMIDA-treated mice, the radioactivities were high in kidney, bone, and Harder's gland; moderate in lung, liver, and blood. Remarkable accumulation of ^{63}Ni was found in bone, and reduced in lung and skin (Fig 1B).

At 8 h, in general, radioactivities were reduced in tissues after iv $^{63}NiCl_2$. The radioactivities were moderate in kidney, lung, blood, and urine, and low in bone, intestine, skin, and liver (Fig 1C). In CBMIDA-treated mice, the levels were increased in kidney, bone, intestine, and skin, but remarkably decreased in lung and blood (Fig 1D).

DISCUSSION

Antidote principle of chelating agent was to combine with metal ion to form complex with a lower toxicity, it reduced the metal ion to the target organs poisoned or enhanced the urinary excretion of the ion. The present study showed that CBMIDA markedly raised the survival rate of $NiCl_2$-poisoned mice and rats, but the excretion of ^{63}Ni through urine and feces was not increased at 24 h. The detoxificating effect of CBMIDA on nickel might be that the chelate was combined with nickel, to form complex of lower toxicity in body.

The tissue distribution and localization of ^{63}Ni were similar. The ^{63}Ni deposited in organs showed as the reports[6], whereas given CBMIDA is more effective for removing nickel from lung, but to accumulate in bone. The metabolism of CBMIDA was mainly deposited in bone except the excretion[7]. It might be that CBMIDA formed the complex of CBMIDA-Ni, and enhanced the ^{63}Ni content in bone due to its action in affinity bone.

In conclusion, CBMIDA markedly raised survival rate of nickel-poisoned mice and rats and decreased content of ^{63}Ni in lung and blood.

REFERENCES

1 Nieboer E, Rossetto FE, Menon CR. Toxicology of nickel compounds. In: Sigel H, Sigel A, editors Metal ions in biological systems: vol 23. Nickel and its role in biology. New York: Marcel Dekker; 1988. p 359 – 402.

2 Tao ZQ, Xu XH, Yan XM, Chen ZJ, Zhang JS, Liang YY, *et al*. Pharmacological studies on catecholamic acid, an efficient chelating agent for metal mobilization. Plzen lek Sborn 1988; 56 Suppl: 95 – 7.

3 Tao ZQ, Xu XH, Yan XM, Chen ZJ, Zhang JS, Liang YY, *et al*. Detoxication and mobilization of uranium by catecholamic acid. Acta Pharmacol Sin 1987; 8: 284 – 8.

4 Fukuda S, Inda H, Hseih YY, Chen WZ. Effect of CBMIDA [catechol-3, 6-bis (methyleiminodiacetic acid)] on removal of plutonium in rats. Hoken Butsuri 1992; 27: 11 – 5.

5 Waddekk WJ, Marlowe C. Autoradiography. In: Garrett ER, Hirtz JL, editors. Drug fate and metabolism: methods and techniques; vol 1. 1st ed. New York: Marcel Dekker; 1977. p 1 – 25.

6 Oskarsson A, Tjälve H. Autoradiography of nickel chloride and nickel carbonyl in mice. Acta Pharmacol Toxicol 1977; 41 Suppl I: 158 – 9.

7 Zhang JS, Liang YY, Yan XM. Whole-body autoradiographic localization of catecholamic acid in mice. Acta Pharmacol Sin 1994; 15: 79 – 80.

80-84 (21)

双酚胺酸对小鼠和大鼠氯化镍解毒及分布的影响[1]

严雪铭[2], 陶正琴, 梁献毅, 陈振家, 张建时, 徐新华 (中国科学院上海药物研究所, 上海 200031, 中国)

R595.2

关键词 螯合剂; 双酚胺酸; 镍; 半数致死量; 放射自显影术; 组织分布

重金属中毒

目的: 研究双酚胺酸(CBMIDA)对氯化镍的解毒作用. **方法**: $NiCl_2$ 中毒后, 立即给予 CBMIDA, 记录动物存活数; 小鼠 iv $^{63}NiCl_2$后给药, 测定 24 h 组织中^{63}镍; 用整体放射自显影术, 显示小鼠体内^{63}镍分布. **结果**: sc CBMIDA 0.5 – 1.5 $g \cdot kg^{-1}$ 对 ip $NiCl_2$ 500 $mg \cdot kg^{-1}$有解毒作用; 小鼠 ip $NiCl_2$ LD_{50}为 82.8 $mg \cdot kg^{-1}$, 给药 1.5 或 2.5 $g \cdot kg^{-1}$, LD_{50}分别为 789 和 820 $mg \cdot kg^{-1}$; 大鼠 im CBMIDA 500 $mg \cdot kg^{-1}$使 $NiCl_2$ 的 LD_{50}提高 8 倍; 组织中^{63}镍测定和定位显示, CBMIDA 减少肺和血液中^{63}镍, 增加了骨中^{63}镍, 24 h 尿、粪^{63}镍排出与对照组无明显差异. **结论**: CBMIDA 有效地解除镍毒性, 提高动物存活率, 降低镍在肺部的滞留.