BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Sep; 17 (5): 415-418

# Pharmacokinetics of germanium after *po* β-carboxyethylgermanium sesquioxide in 24 Chinese volunteers

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**KEY WORDS** germanium; β-carboxyethylgermanium sesquioxide; pharmacokinetics; atomic absorption spectrophotometry

**AIM**: To compare the pharmacokinetics after *po* different doses of β-carboxyethylgermanium sesquioxide (Ge-132). **METHODS**: An atomic absorption spectrophotometric system was used to measure germanium concentrations in plasma and urine samples after *po* Ge-132 1 (low dose, LD), 2.5 (medium dose, MD), and 4 (high dose, HD) $g \cdot m^{-2}$ in 24 healthy volunteers (one dose per 8 subjects). **RESULTS**: $T_{\frac{1}{2}\alpha}$ (LD, 1.2 ± 0.7 h; MD, 1.1 ± 0.6 h; HD, 1.2 ± 0.5 h), $T_{\frac{1}{2}\beta}$ (LD, 5.2 ± 1.2 h; MD, 5.8 ± 2.5 h; HD, 5.5 ± 1.4 h) and *Cl/F* (LD, 33 ± 12 $L \cdot h^{-1}$; MD, 35 ± 10 $L \cdot h^{-1}$; HD, 33 ± 11 $L \cdot h^{-1}$) were not dose-related. $T_{max}$ was between 0.75 h and 2 h. $C_{max}$ (LD, 5.3 ± 2.2 $mg \cdot L^{-1}$; MD, 13 ± 5 $mg \cdot L^{-1}$; HD 18 ± 8 $mg \cdot L^{-1}$, HD) and AUC (LD, 31 ± 13 $mg \cdot h \cdot L^{-1}$; MD, 60 ± 16 $mg \cdot h \cdot L^{-1}$; HD, 79 ± 42 $mg \cdot h \cdot L^{-1}$) were positive correlation to the dose of Ge-132. Urine-eliminated germanium within 24 h accounted for 11 ± 3 % of LD, 9 ± 3 % of MD, and 6 ± 5 % of HD (calculated from Ge/F) and showed a negative correlation to the dose. **CONCLUSION**: 1) Intracorporal process of Ge after *po* Ge-132 coincided with the first-order absorption and elimination with two-compartment kinetic model; 2) The amount of germanium eliminated in urine was below 11 %.

β-Carboxyethylgermanium sesquioxide (Ge-132) is not only an immunomodulator[1], but also a low toxic anticancer agent[2,3]. Ge-132 promoted growth of cell and nucleic acid synthesis[4], facilitated secretion of acidophilic cell of hypophysis in cell culture[5], improved the metabolism of cultured neonatal rat myocytes and protected myocytes from isoproternol-induced injury[6], and scavenged free radicals[7]. Pharmacokinetic character of *po* a small dose (0.1 g) of Ge-132 in humans fitted well to one-compartment model and first-order kinetics through determination of germannium (not prototype) in urine with spectrophotometry of phenylfluorones[8], whereas in rabbits was two-compartment[9]. This experiment was a part of phase I clinical trial in treatment of cancer. Doses used in this study underlay the tolerance test of Ge-132 in humans. This experiment was to study the pharmacokinetic rule by comparison of *po* a series doses of Ge-132 in humans and provide reasonable clinical therapeutic schedule.

Received 1994-11-07 Accepted 1996-04-09

## MATERIALS AND METHODS

**Drugs** Ge-132 oral liquid (synthesized by Guangzhou Research Institute of Semiconductor Materials, dissoluted in distilled water and adjusted by NaOH solution to physiological pH) contained Ge-132 100 $g \cdot L^{-1}$ (lot No: 940520). Standard liquid of germanium dioxide ($GeO_2$), which contained Ge 1 $mg \cdot L^{-1}$, was kindly provided by Kanto Chemical Co Ltd, Japan (lot No: CR-0-01-07).

**Instruments and operation conditions** A graphite furnace atomic absorption spectrometer (Model Z/3030) was used, λ 265.1 nm, slit 0.7, the dryness in the 1st slope temperature-raising at 130 ℃ for 10 s with retention for 25 s, the 2nd at 170 ℃ for 10 s with retention for 10 s, the incineration at 1200 ℃, the atomization at 2000 ℃, and the ablution at 2300 ℃.

**Protocol** A total of 24 (12 M, 12 F) volunteers (23 ± *s* 3 a, weighing 53 ± *s* 7 kg, the Han nationality) were judged to be healthy based on complete physical examination, ECG, blood chemistry and urine analysis. No subject exposed to any other medicine throughout the test and 1 month before. Ge-132 doses were 1 (low dose), 2.5 (medium dose), and 4 (high dose) $g \cdot m^{-2}$ body surface area. The volunteers were randomly divided into 3 groups of 8 (4 M, 4 F) subjects each. One of the 3 doses of Ge-132 was single and orally administrated to each group. Blood samples (3 mL) were drawn from an antecubital vein at the following times: before, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 6, 9,

12, and 24 h after medication. Urine samples were collected before dosing and during hours 0 – 6, – 12, and – 24 h.

**Preparation of calibration curves** Trichloroacetic acid 5 % 0.5 mL was added to 0.5 mL of Ge-132 10, 25, 50, 75, 100 $\mu g \cdot L^{-1}$ plasma and blank plasma. The mixture was vortexed for 30 s, and subsequently centrifuged at 1200 × *g* for 10 min. Supernatant fluid was used for detection. Formic acid 1 mL was added to 1 mL of urine containing the same amount of Ge-132 in plasma and blank urine. The mixture was digested for 6 h, diluted in distilled water and detected. We entrusted Chinese National Analytical Center, Guangzhou, with responsibility for measuring Ge in biological samples.

**Calculation** The data were processed by computer program 3P87 provided by China Pharmaceutical Association (Zhang WG, Yang YC, Tang ZM, Liu X, Sun RY, Yu ZL. "3P87" a calculating program of practical pharmacokinetics. Infor Chin Pharmacol Soci 1988; **5** (4): 19). Program of equation of two-compartment with first-order absorption and elimination was used for calculation. Weigh parameter was 1/C. Statistical moment was applied to calculate AUC.

## RESULTS

**Calibration curves** The coefficients of variation within-day and between-day were <5 % ($n$ = 11) and 5.5 % ($n$ = 9), respectively. Recovery was 91.2 – 97.0% ($n$ = 8) in plasma samples and 97.2 – 105.6 % ($n$ = 7) in urine samples. Characteristic quantity was 43 ng · $L^{-1}$. The calibration curves of Ge in plasma and urine were linear over the range 10 – 100 mg·$L^{-1}$. The linear regression equations were

Plasma: $C = 0.00050 + 0.0021A$

($r = 0.9997$; $n = 5$)

Urine: $C = 0.00066 + 0.0023A$

($r = 0.9993$; $n = 5$)

Here, $C$ standed for concentration and $A$ represented peak area.

**Pharmacokinetic characteristics of Ge** Concentration-time curves after ingestion of Ge-132 1, 2.5, and 4 g · $m^{-2}$ coincided with first-order absorption and elimination model (Fig 1).

$T_{max}$ was between 0.7 h and 2 h for the 3 dosages. $C_{max}$ and AUC were directly correlated to the doses. $T_{\frac{1}{2}\alpha}$, $T_{\frac{1}{2}\beta}$, and $Cl/F$ were irrespective of the doses. AUC and $C_{max}$ were directly correlation to Ge-132 doses. Ge eliminated in urine within 24 h after oral ingestion of Ge-132 accounted for 11 ± 3 % (173 ± 64 mg) of low dose, 9 ± 3 % (266 ± 90 mg) of medium dose, and 6 ± 5 % (237 ± 205 mg) of high dose (Tab 1).

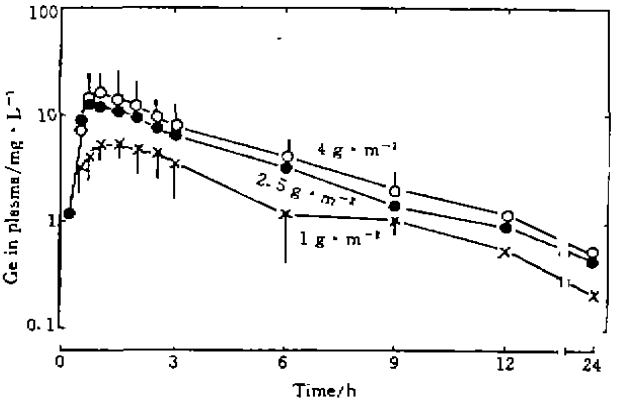


**Fig 1. Ge concentrations in plasma after *po* Ge-132 oral liquid.**

**Tab 1. Pharmacokinetic parameters of Ge-132 in 24 healthy volunteers after *po* Ge-132 1, 2.5, and 4 g · $m^{-2}$ ($n$ = 8 volunteers in each group, $\bar{x} \pm s$).**

| Ge-132 | 1 g·$m^{-2}$ | 2.5 g·$m^{-2}$ | 4 g·$m^{-2}$ |
|---|---|---|---|
| $T_{\frac{1}{2}\alpha}$, h | 1.2±0.7 | 1.1±0.6 | 1.2±0.5 |
| $T_{\frac{1}{2}\beta}$, h | 5.2±1.2 | 5.8±2.5 | 5.5±1.4 |
| $K_a$, $h^{-1}$ | 1.6±0.5 | 2.8±1.8 | 2.8±2.7 |
| $K_{21}$, $h^{-1}$ | 0.9±1.2 | 0.3±0.2 | 0.23±0.08 |
| $K_{10}$, $h^{-1}$ | 0.31±0.07 | 0.4±0.1 | 0.4±0.1 |
| $K_{12}$, $h^{-1}$ | 0.3±0.3 | 0.3±0.2 | 0.2±0.2 |
| $V_c$, L·$m^{-2}$ | 131±64 | 151±87 | 343±478 |
| $T_{max}$, h | 1.3±0.3 | 1.0±0.3 | 1.3±0.3 |
| $C_{max}$, mg·$L^{-1}$ | 5.3±2.2 | 13±5 | 18±8 |
| MRT, h | 5±3 | 5.2±0.5 | 5.5±1.5 |
| $Cl/F$, L·$h^{-1}$ | 33±12 | 35±10 | 33±11 |
| AUC, mg·h·$L^{-1}$ | 31±13 | 60±16 | 79±42 |

## DISCUSSION

Although $T_{max}$ was between 0.75 h and 2 h at 3 Ge-132 groups, $T_{max}$ in the majority of volunteers (21/24) was between 0.75 h and 1 h. Ge peak concentration at the 3 dosage groups was 5.7±2.1, 12.6±5.7, and 18.2±8.2 g·$L^{-1}$, respectively. $C_{max}$ was 3.4 – 7.4 mg·$L^{-1}$ at the group of 1 g ·$m^{-2}$ of Ge-132, 7.30 – 19.8 mg·$L^{-1}$ at 2.5 g ·$m^{-2}$ group and 1.5 – 32.5 mg·$L^{-1}$ at 4 g·$m^{-2}$

group, and 2.6-fold in $C_{max}$ over $C_{min}$ was shown at low dosage group, 2.8-fold at medium dosage group and tens of times at high dose group. No anticancer action of Ge-132 *in vitro* was found. Relationship between peak concentration of Ge-132 and anticancer action *in vivo* has not yet been reported and need to be studied further. The ratio of $C_{max}/C_{min}$ was 5.8-fold in one case and 24.4-fold in another case at HD group. $K_a$ values became an increase as the dose of Ge-132 ($1-2.5\ g\cdot m^{-2}$), but $K_a$ of $4\ g\cdot m^{-2}$ group did not increase compared with $2.5\ g\cdot m^{-2}$ group and the standard deviation of $K_a$ of $4\ g\cdot m^{-2}$ group was large. The data showed that absorption after *po* $1-2.5\ g\cdot m^{-2}$ of Ge-132 was relatively regular, whereas absorption of *po* Ge-132 $4\ g\cdot m^{-2}$ was fairly individual difference and blood concentration of Ge was more undulate. Half-life of absorption phase ($T_{\frac{1}{2}\alpha}$) and elimination phase ($T_{\frac{1}{2}\beta}$) were irrespective of *po* the dose of Ge-132. $C_{max}$ positively related to oral dose of Ge-132 ($r=0.9981$). $Cl$, however, was not a constant in 3 doses of Ge-132 and also did not become small as dosage increase of Ge-132 like non-linear pharmacokinetic model. The phenomenon of $Cl$ was associated with AUC. AUC ratio of 3 dosage groups ($31:60:79\ mg\cdot h\cdot L^{-1}=1:1.9:2.5$) was not identical with dosage ratio of Ge-132 ($1:2.5:4\ g=1:2.5:4$), although AUC was directly correlation to dose of Ge-132 ($r=0.9944$). That is to say a factor of $F$ (bioavailability) influenced $Cl$. $Cl/F$ instead of $Cl$ was expressed as clearance to avoid having an effect of the unidentity between AUC ratio and dose ratio on $Cl$. Thus, $Cl/F$ was close to a constant. The data illustrated intracorporal process of *po* Ge-132 coincided with first-order kinetics and non-linear kinetics was not seen after *po* Ge-132 of $1-4\ g\cdot m^{-2}$ because of unchange of half-life and $Cl/F$ as dosage increase and postivitve correration of $C_{max}$ to dose of Ge-132. Eliminating ratio of urine Ge was calculated by a formula (actual urine-eliminated percentage of Ge/$F$) based on the influence of $F$. Ge eliminated in urine within 24 h after oral ingestion accounted for $11\pm3$ % of low dose, $9\pm3$ % of medium dose, and $6\pm5$ % of high dose. The data was similar to about 10 % of Ge (average 109.12 mg) elimilated in 24-h urine after *po* 100 mg of Ge-132[8], suggested that renal elimination of Ge after *po* Ge-132 be fairly low. Other eliminatiing ways of Ge-132, such as feces, bile, and so forth, might exist. Defect of either atomic absorption spectrophotometry or spectrophotometry of phenylgluorones was determination of germanium rather than Ge-132 prototype. The methods could not reflect real level of Ge-132, especially metabolites that dissociated with Ge. That might be one reason of low level of Ge in urine.

We concluded from all the data: 1) absorpting and eliminating process of *po* 1, 2.5, and $4\ g\cdot m^{-2}$ of Ge-132 coincided with the first-order and two-compartment kinetic model; (2) Ge eliminated by kidneys was below 11 % of *po* Ge-132; (3) as far as half-life was concerned, Ge-132 was given twice daily orally may be suitable for therapy of cancer.

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## 口服β-羧乙基倍半锗氧化物后锗在24名中国人体内的药物动力学

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关键词 锗；β-羧乙基倍半锗氧化物；药物动力学；原子吸收分光光度测定法

目的：比较口服不同剂量β-羧乙基倍半锗氧化物(Ge-132)的药物动力学的规律. 方法：三组健康志愿者(每组8人)分别一次口服Ge-132口服液1(低剂量，LD)、2.5(中剂量，MD)、4(高剂量，HD) $g \cdot m^{-2}$，用原子吸收光谱法测定24 h血浆锗和尿锗浓度，比较不同剂量Ge-132的人体药动学. 结果：用药后，$T_{\frac{1}{2}\alpha}$(LD，1.2±0.7 h；MD，1.1±0.6 h；HD，1.2±0.5 h)、$T_{\frac{1}{2}\beta}$(LD，5.2±1.2 h；MD，5.8±2.5 h；HD，5.5±1.4)和 $Cl/F$ (LD，33±12 $L \cdot h^{-1}$；MD，35±10 $L \cdot h^{-1}$；HD，33±11 $L \cdot h^{-1}$)与剂量无关. $T_{max}$在0.75 h和2 h之间. $C_{max}$(LD，5.3±2.2 $mg \cdot L^{-1}$；MD，13±5 $mg \cdot L^{-1}$；HD，18±8 $mg \cdot L^{-1}$)和AUC (LD，31±13 $mg \cdot h \cdot L^{-1}$；MD，60±16 $mg \cdot h \cdot L^{-1}$；HD，79±42 $mg \cdot h \cdot L^{-1}$)与剂量呈正相关. 24 h尿锗排出量占所给低剂量的11±3 %、中剂量的9±3 %、高剂量的6±5 %(用实际尿锗排出百分数/F的公式计算)，与剂量呈负相关. 结论：口服Ge-132后符合一级吸收和一级消除二房室模式；经肾脏排泄的锗约占口服量的11 %以下.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Sep; 17 (5): 418－420

## Inhibitory effects of dextromethorphan on c-*fos* protein expression during focal cerebral ischemia in rats[1]

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**KEY WORDS** cerebral ischemia; proto-oncogene proteins c-*fos*; immunohistochemistry; dextromethorphan

**AIM:** To study the effect of dextromethorphan (DM) in focal cerebral ischemia. **METHODS:** The c-*fos* protein was detected immunohistochemically in the brain of rats after focal cerebral ischemia (induced by placing a nylon thread in the lumen of the internal carotid artery) with and without treatment with DM. **RESULTS:** Focal cerebral ischemia induced c-*fos* protein expression outside the core territory of the middle cerebral artery (MCA) and neuronal damage in the core territory of the MCA. There was an evident expression of c-*fos* protein in the ipsilateral regions outside the MCA territory (eg cingulate cortices, piriform cortices and entorhinal cortices), and in the contralateral regions of hippocampus after 4-h reperfusion following 1-h MCA occlusion. But morphological results showed severe edema and neuronal damage in the core territory and the ipsilateral hippocampus. DM blocked both the c-*fos* protein induction and neuronal damage in all regions. **CONCLUSION:** DM reduced c-*fos* protein expression and blocked the neuronal damage after focal cerebral ischemia.

Proto-oncogene proteins c-*fos* can be induced by a variety of stimili, such as elevation of intracellular calcium level, exposure to excitatory amino acid *in vitro*, and glutamate receptor agonist *in vivo*[1-3]. Local devascularization of cerebral cortex and global ischemia result in transient c-*fos* expression which is blocked by *N*-methyl-*D*-aspartate (NMDA) receptor antagonist[4-6]. Focal

[1] Supported by Key Projects for the Eighth Five-year Plan, № 8591307.
Received 1994-05-03 Accepted 1995-12-29