arrhythmias are generated. The drug concentrations are expected to be higher in groups in which the drug studied is administered prior to occlusion or on reperfusion than that administered during the occlusion period.

The classification of the antiarrhythmic agent $MI_2$ could also be evaluated by the slowing of intraventricular impulse conduction and listed into the Ic group[7,8] same as changrolin.

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## 常咯啉的6个吲哚衍生物的抗心律失常活性


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**提要** 以抑制豚鼠哇巴因诱发室速而转为窦律的维持时间，比较心律失常的强度. 3',5'-哌啶基的 $MI_2$ 最强(>60 min)，比常咯啉(25 min)强 2.4 倍. 抗胆碱作用亦减弱. 在不同时间给药，比较对再灌诱发心律失常的抑制作用，在再灌的即刻或结扎前 30 min 给药，抑制作用最强. 由于抑制室内传导，$MI_2$ 可能属于 Ic 类.



**关键词** 吲哚类；抗心律失常药；噻唑啉类；心肌梗死；心室纤颤



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# Effects of intratracheal instillation of fenvalerate on the ultrastructures of pulmonary alveolar macrophages in rat


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**ABSTRACT** On 1 d after instillation of fenvalerate (Fen) 0.19, 0.93, 4.66, and 23.3 mg · $kg^{-1}$, and on 30 min, 4 h, 1 d, 4 d, and 7 d after instillation of Fen 4.66 mg · $kg^{-1}$ by a single intratracheal instil- lation, respectively, the ultrastructural changes in rat pulmonary alveolar macrophages (PAM) were observed, and the toxicity indices (TI) were calculated. It was found that the ineffective dose of Fen was 0.19 mg · $kg^{-1}$, and the threshold dose was $\leqslant$ 0.93 mg · $kg^{-1}$ as well as the serious



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intoxication dose $\geqslant$4.66 mg · kg$^{-1}$. The results suggest that Fen is quickly toxic to rat lungs and quickly biodegraded in the lungs.

**KEY WORDS** valerates; irrigation; bronchi; pulmonary alveoli; macrophages; electron microscopy; toxicology; pesticides

Fenvalerate [Fen, (R,S)–α–cyano–3–phenoxybenzyl(R,S)–2–(4–chlorophenyl)–3–methylbutyrate] is a synthetic pyrethroid pesticide which possesses broad spectrum and high insecticidal activity, low mammalian toxicity and low residue in the biosphere. It has been widely used to control insects and many insect vectors of disease in agriculture, forestry, livestock breeding, household, stored products, and horticulture. Although Fen is not found to have any deleterious effect such as carcinogenicity, teratogenicity, and mutagenicity[1], its adverse effects have been reported on respiratory system of the workers exposed to its wettable powder in the field[2] and its vapour in the air of worshop[3]. However, the mechanisms of Fen toxicity and its metabolism in lungs have not been yet reported in literature. The present study was to find the ineffective and threshold doses of Fen, to observe its toxic effects and investigate the mechanism of intoxication.

## METHODS

Forty Sprague–Dawley rats, ♂, weighing 196± SD 15 g, provided by Animal Research Center of Nanjing Medical College, were at random devided into 10 groups. The technical grade of Fen composed of 90.5% active ingredient was obtained from Jiangsu Institute of Hormone. A single intratracheal instillation of Fen was performed at 0.19, 0.93, 4.66, and 23.3 mg · kg$^{-1}$, respectively. The rats were sacrificed on 30 min, 4 h, 1 d, 4 d, and 7 d after drug instillation, and were bronchoalveolar lavaged[4,5]. The preparation of PAM for transmission electron microscope (Philips EM–400, Holland) was the same as that mentioned in reference (6). The ultrastructural parameters of PAM were calculated by means of coherent multipurpose test system and coherent double lattice test system ($n$=36 cells). Data were analysed by ANOVA.

## RESULTS

**Changes in two–dimensional images** On 1 d after the instillation of Fen and on the different time after instillation of Fen 4.66 mg · kg$^{-1}$, the changes in PAM two–dimensional images were shown in Fig 1. PAM membranes were seriously damaged at doses of $>$ 4.66 mg · kg$^{-1}$ (Fig 1 A–D, Plate 1). The common features of the damaged were: 1) PAM plasma membranes were broken, Golgi area (Go) was partly broken and even disappeared, part of lysosomal membrane was split (Fig 1 C), some of lipid droplets (LD) were accumulated in primary lysosomes (PLy) (Fig 1 A) and others scattered in cytoplasm (Fig 1 B, Plate 1). 2) Cytoplasm, full of residual bodies (RB), secondary lysosomes (SLy), and free ribosomes (FR) which fell off from rough endoplasmic reticulum (RER), displayed a number of lamellar bodies (LB) (Fig 1 A, B, Plate 1). 3) Mitochondria (Mi), RER, and smooth endoplasmic reticulum (SER) were conspicuously reduced in number, And the expansion was shown in their membrane structures. A number of Mi indicated cristae lysis, matrix swelling, and vacuolization (Fig 1 D, Plate 1). Fen 0.93 mg · kg$^{-1}$ only induced cristae lysis or matrix swelling in some of Mi, while the other submicrostructures did not display any abnormal changes (Fig 1 E, Plate 1). Fen 0.19 mg · kg$^{-1}$ had no effect on PAM ultrastructures. On 30 min after instillation, cytoplasm indicated the shrunk Go, many of PLy, a small number of large heterolysosomes (HLy) which phagocytized Fen microparticles, and a

small number of large autolysosomes (ALy) which phagocytized PLy or Mi (Fig 1 F, Plate 1). On 4 h after instillation, cytoplasm showed a number of large HLy like the insect-eaten materials and vacuolated Mi (Fig 1 G, Plate 1). On d 1 after instillation, the changes in PAM submicrostructures were shown in Fig 1 A-D. On d 4 after instillation, the developed Go and a large number of PLy and SLy appeared in cytoplasm. Besides, numbers of Mi, RER, and SER were obviously increased (Fig 1 H, Plate 1). On d 7 after instillation, PAM ultrasturcutres returned to normal.

**Changes in three-dimensional structures**

Morphometry or stereology allows the volume, surface area, number, and lineal extent of the structures in three-dimensions to be assessed from the two-dimensional images of TEM. In this paper, volume density (Vvi) was the total volume of a component i within the total cytoplasmic volume of PAM in section. Surface density (Svi) was the surface area of a component i per unit cytoplasmic volume. Numerical density per unit area (Nai) was the profile number of a particle i within the cytoplasmic area. Numerical density (Nvi) was the number of a particle i within the total cytoplasmic volume. Specific surface ($\delta i$) was the ratio of surface area to volume fraction of a component i. Total area of outer membrane $(Si)_0$ was the product of outer membranous surface density $(Svi)_0$ and average cytoplasmic volume (Vp) (Tab 1, Fig 2).

**Tab 1. Ultrastructural parameters of mitochondria (Mi), lysosome (Ly), and lipid droplet (LD) in pulmonary alveolar macrophages from the rats on 1 d after instillation of fenvalerate (Fen).** $n=36$ cells. $\bar{x} \pm SD$. Mi: $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control and 0.19 mg · kg$^{-1}$ group. $^{+}P>0.05$, $^{++}P<0.05$ *vs* 23.3 mg · kg$^{-1}$. Ly: $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control and 0.19 mg · kg$^{-1}$ group. $^{+}P>0.05$ *vs* 0.93, 4.66, and 23.3 mg · kg$^{-1}$ group. $^{++}P<0.05$ *vs* 23.3 mg · kg$^{-1}$ group. LD: $^{*}P>0.05$, $^{**}P<0.05$ *vs* control, 0.19, and 0.93 mg · kg$^{-1}$ group. $^{+}P>0.05$ *vs* 23.3 mg · kg$^{-1}$ group. $^{++}P<0.05$ *vs* control and 23.3 mg · kg$^{-1}$ group.

| Fen, mg · kg$^{-1}$ | | Vvi, $\mu m^3/\mu m^3$ | Svi, $\mu m^{-1}$ | $\delta i$, $\mu m^{-1}$ | Nai, $\mu m^{-2}$ | Nvi, $\mu m^{-3}$ | $10^{-4} \times (Si)_0$, $\mu m^2$ |
|---|---|---|---|---|---|---|---|
| Control | Mi | 10.3 ± 2.6 | 19.8 ± 3.6 | 1.91 ± 0.25 | 0.63 ± 0.15 | 0.21 ± 0.04 | 2.23 ± 0.41 |
| | Ly | 16.3 ± 2.7 | 20.0 ± 3.4 | 1.19 ± 0.24 | 1.26 ± 0.35 | 0.28 ± 0.06 | 2.25 ± 0.53 |
| | LD | 1.7 ± 0.6 | 3.1 ± 0.9 | 1.82 ± 0.42 | 0.07 ± 0.02 | 0.09 ± 0.03 | – |
| 0.19 | Mi | 10.2 ± 3.4$^{*}$ | 20.3 ± 4.2$^{*}$ | 1.97 ± 0.27$^{*}$ | 0.57 ± 0.21$^{*}$ | 0.19 ± 0.04$^{*}$ | 2.55 ± 0.58$^{*}$ |
| | Ly | 18.4 ± 3.6$^{*}$ | 25.9 ± 3.2$^{*}$ | 1.44 ± 0.32$^{**+}$ | 1.32 ± 0.42$^{*}$ | 0.39 ± 0.08$^{*}$ | 3.25 ± 0.75$^{*}$ |
| | LD | 2.2 ± 1.0$^{*}$ | 3.9 ± 0.9$^{*}$ | 1.75 ± 0.47$^{*}$ | 0.11 ± 0.03$^{*}$ | 0.11 ± 0.03$^{*}$ | – |
| 0.93 | Mi | 7.9 ± 2.2$^{**++}$ | 16.3 ± 3.8$^{*}$ | 2.05 ± 0.34$^{*}$ | 0.49 ± 0.19$^{*}$ | 0.16 ± 0.03$^{*}$ | 2.06 ± 0.44$^{*}$ |
| | Ly | 22.6 ± 4.7$^{**+}$ | 32.3 ± 4.5$^{**+}$ | 1.42 ± 0.39$^{**}$ | 1.43 ± 0.46$^{**+}$ | 0.59 ± 0.17$^{**++}$ | 4.08 ± 0.86$^{**++}$ |
| | LD | 3.2 ± 1.3$^{++}$ | 5.3 ± 2.0$^{++}$ | 1.70 ± 0.49$^{*}$ | 0.10 ± 0.4$^{*}$ | 0.13 ± 0.02$^{*}$ | – |
| 4.66 | Mi | 6.3 ± 1.8$^{***+}$ | 7.7 ± 1.7$^{**+}$ | 1.22 ± 0.31$^{**+}$ | 0.39 ± 0.12$^{***+}$ | 0.08 ± 0.03$^{***+}$ | 1.04 ± 0.32$^{***+}$ |
| | Ly | 23.3 ± 4.5$^{**}$ | 35.2 ± 5.3$^{**}$ | 1.52 ± 0.42$^{**}$ | 1.68 ± 0.55$^{**}$ | 0.70 ± 0.27$^{**+}$ | 4.77 ± 1.04$^{**++}$ |
| | LD | 4.2 ± 1.9$^{**+}$ | 8.8 ± 3.6$^{**+}$ | 2.07 ± 0.62$^{*}$ | 0.19 ± 0.06$^{**+}$ | 0.32 ± 0.09$^{**+}$ | – |
| 23.3 | Mi | 5.5 ± 1.8$^{***}$ | 6.9 ± 1.8$^{***}$ | 1.25 ± 0.38$^{**}$ | 0.33 ± 0.14$^{***}$ | 0.07 ± 0.02$^{***}$ | 0.96 ± 0.36$^{***}$ |
| | Ly | 25.1 ± 6.3$^{**}$ | 37.9 ± 4.9$^{**}$ | 1.55 ± 0.48$^{**}$ | 1.75 ± 0.51$^{**}$ | 0.84 ± 0.20$^{***}$ | 5.27 ± 1.12$^{***}$ |
| | LD | 4.6 ± 2.2$^{**}$ | 9.4 ± 3.0$^{**}$ | 2.04 ± 0.58$^{*}$ | 0.23 ± 0.06$^{**}$ | 0.34 ± 0.05$^{**}$ | – |

Note: Vvi — volume density. Svi — surface density. $\delta i$ — specific surface. Nai — numerical density per unit area. Nvi — numerical density. $(Si)_0$ — total area of outer membrane.

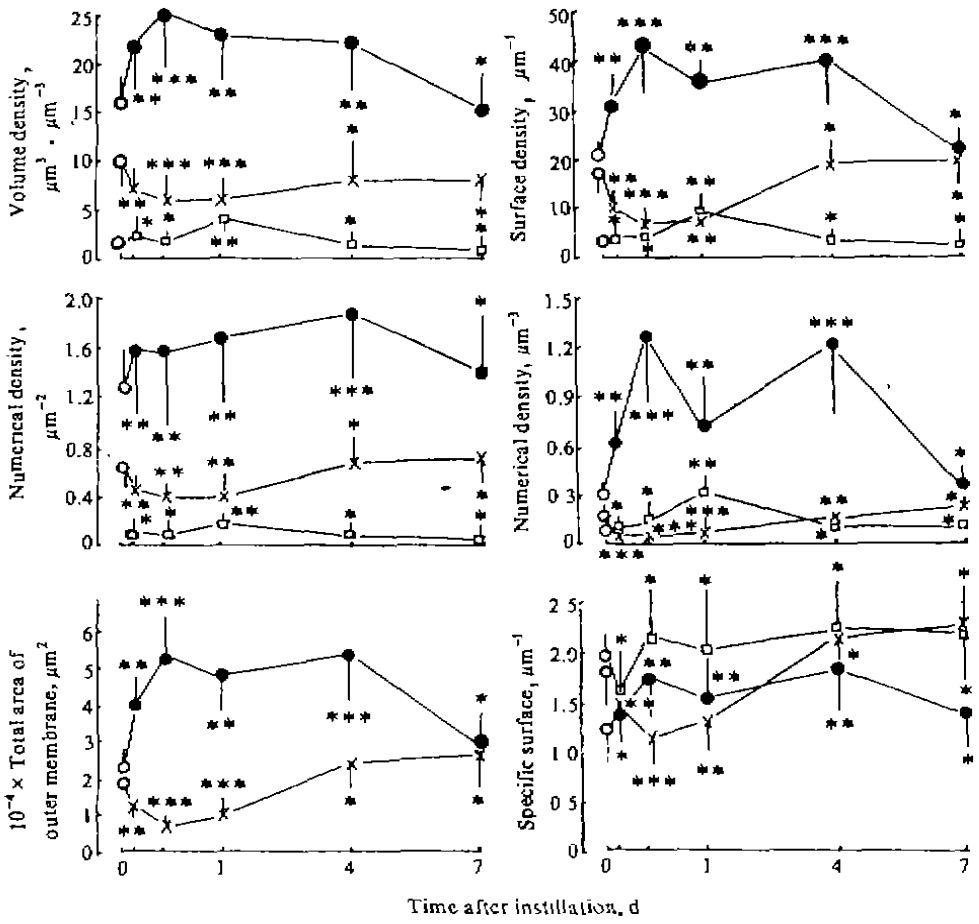


**Fig 2. Ultrastructural changes in pulmonary alveolar macrophages from the rats on the different time after a single intratracheal instillation of fenvalerate (Fen) 4.66 mg · kg$^{-1}$. $n$=36 cells for control (○), lysosome (●), mitochondria (×), and lipid droplet (□). $\bar{x}\pm$SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control group.**

**Changes in toxicity index (TI)** TI was the toxicity ratio of the exposure group to the control group. The formula of TI was: $TI=\Sigma[Wi(Ei/Ci)]^{*}$. Ei and Ci indicate the detected values of the selected indicators from the exposed and control groups, respectively. Wi expresses the weighting coefficient determined in light of the origins and biological significances of the selected indicators. $^{*}Mi=Ci/Ei$. The results of PAM TI calculated from Ly, LD, and Mi were presented in Tab 2.

## DISCUSSION

According to the changes in two-dimensional images, submicrostructural morphometry parameters, and TI of PAM after instillation of Fen, we found that Ly and LD increased with logarithmic exposure dose, but Mi, RER, SER, and Go showed decreased tendencies. The fact showed that changes in PAM submicrostructures exhibited dose-dependent relationships to some extent. According to these data, we conclude that ineffective dose of Fen to PAM is 0.19

**Tab 2. Toxicity index (TI) of pulmonary alveolar macrophages from rats 1 d after instillation of fenvalerate (Fen) and at different times after instillation of Fen 4.66 mg · kg$^{-1}$. $n$ = 36 cells.**

| Fen, mg · kg$^{-1}$ | Lysosome Ei / Ci | Wi | Wi(Ei/Ci) | Lipid droplet Ei / Ci | Wi | Wi(Ei/Ci) | Mitochondria Ci / Ei | Wi | Wi(Ci/Ei) | TI |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.19 | 2.65 | 0.16 | 0.42 | 2.48 | 0.02 | 0.05 | 2.04 | 0.10 | 0.20 | 0.66 |
| 0.93 | 3.65 | 0.16 | 0.57 | 3.15 | 0.02 | 0.06 | 2.47 | 0.10 | 0.24 | 0.89 |
| 4.66 | 4.21 | 0.16 | 0.68 | 6.36 | 0.02 | 0.13 | 5.08 | 0.10 | 0.51 | 1.32 |
| 23.3 | 4.79 | 0.16 | 0.78 | 6.81 | 0.02 | 0.14 | 5.76 | 0.10 | 0.58 | 1.49 |
| After instillation | | | | | | | | | | |
| 30 min | 3.78 | 0.16 | 0.60 | 2.42 | 0.02 | 0.05 | 5.30 | 0.10 | 0.53 | 1.19 |
| 4 h | 6.66 | 0.16 | 1.06 | 3.02 | 0.02 | 0.06 | 6.88 | 0.10 | 0.69 | 1.81 |
| 1 d | 4.21 | 0.16 | 0.68 | 6.36 | 0.02 | 0.13 | 5.08 | 0.10 | 0.51 | 1.32 |
| 4 d | 6.17 | 0.16 | 0.99 | 2.06 | 0.02 | 0.04 | 2.51 | 0.10 | 0.25 | 1.27 |
| 7 d | 2.16 | 0.16 | 0.35 | 1.58 | 0.02 | 0.03 | 1.99 | 0.10 | 0.20 | 0.57 |

mg · kg$^{-1}$, and threshold dose is 0.93 mg · kg$^{-1}$, which further verifies the conclusion we had made previously[3]. In view of PAM as the major means of detoxifying inhaled industrial chemicals, and its functional conditions which effectively reflect the physiologic and pathologic states of lungs, the result is one of the scientific bases for the formulation of Fen hygienic standards.

The damage of lung cells is an important mark of the lung parenchyma injury. On 1 d after instillation of Fen $\geq$ 4.66 mg · kg$^{-1}$, PAM cytoplasm showed a large number of RB. NvLy (0.70) increased by 1.5 times over control, yet decreased 41% *vs* that of 4 d and 45% *vs* that of 4 h after the instillation. At the same time, NvLD (0.32) was twice as much as that of 4 h and 3 times as much as that of 4 d. The fact indicated that lysosomal membranes had been split (Fig 1 C). We hold that it is not only a direct factor which caused PAM autolysis, but also an important factor which induced the lung tissue injury. Besides, a number of LB which appeared in PAM cytoplasm showed that type II epithelial cells (EP-II) had been damaged.

PAM damage and its recovery are associated with the physical and chemical properties of Fen[7,8]. Fen is a kind of highly lipophilic pyrethroid pesticide containing alpha-cyano. Fen quickly affects the ionic balance and alters the three-dimensional structure of biomembranes when it is in contact with tissue cells. This study support the viewpoit. On 30 min after instillation, the formation of ALy suggested that phagocytosis of SLy had been activated greatly as a result of the injury of Mi and Ly themselves, though increases in PLy, SLy, and HLy were protective responses from Fen. The reason that PAM damages returned to normal on about 4 d later lies in the fact that pyrethroid pesticides in mammals are rapidly biodegraded and the products are quickly excreted because of oxidation by mixed-function oxidases or because of the metabolism of ester cleavage. The rapid recovery feature, of course is also connected with PAM cytokinetic change[9]. The above-mentioned time-response characteristic is of an important referential value in the prophylaxis and treatment of acute Fen intoxication occupationally.

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## 气管灌注氰戊菊酯对大鼠肺泡巨噬细胞超微结构的影响

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**提要** 采用电镜检查和形态学计量技术观察氰戊菊酯(Fen)对肺泡巨噬细胞的毒性，并以毒性指数(TI)评价中毒效应程度。发现 Fen 的无效剂量为 0.19 mg·kg$^{-1}$，阈剂量≤0.93 mg·kg$^{-1}$，≥4.66 mg·kg$^{-1}$ 可导致严重的细胞生物膜损伤。染毒后 4–24 h 为反应高峰期，TI=1.81–1.32，4 d 后恢复正常。结果提示，Fen 具有毒性作用发生快及生物降解快的特点。

**关键词** 戊酸盐类；灌洗术；支气管；肺泡；巨噬细胞；电子显微镜检查；毒理学；农药

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# 二氢埃托啡对离体豚鼠回肠、小鼠和兔输精管电场刺激收缩的影响

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**Effect of dihydroetorphine on contraction response of guinea pig ileum, mouse, and rabbit vas deferens induced by electric field stimulation** *in vitro*

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**ABSTRACT** Dihydroetorphine (DHE) inhibited contraction response of isolated guinea pig ileum and mouse vas deferens induced by electric field stimulation with $IC_{50}$ values of $(4.4 \pm 4.3) \times 10^{-16}$ mol·L$^{-1}$ and $(1.1 \pm 1.4) \times 10^{-18}$ mol·L$^{-1}$ respectively. These effects of DHE were antagonized by naloxone (Nal). On guinea pig ileum, the antagonistic effect of Nal against DHE was similar to those against morphine and etorphine. However, on mouse vas deferens, the antagonistic effect of Nal against DHE was more potent than those against morphine and etorphine. Besides, DHE showed antagonistic effect on k-receptors in isolated rabbit vas deferens.

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**KEY WORDS** dihydroetorphine; morphine; naloxone; ileum; vas deferens