Isolation and Preparation of ^{3}H-tetrandrine

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SUMMARY

Tetrandrine is a bisbenzytehahydroisoquinoline alkaloid. Its structure is very complex and the preparation of labeled derivatives is difficult. We have prepared ^{3}H-tetrandrine using the tritium gas exposure method.[1] This method produces many fragments which make isolation and purification of small quantities difficult. We investigated these fragments of unlabeled d-tetrandrine and found almost all contain one to several hydroxide groups. Using this information, we designed a two-step paper chromatography method for isolation and purification of ^{3}H-Tetrandrine. This method involves the application of a pH 4.7 Na_2HPO_4-citric acid buffer to the paper to block the migration of hydroxide group fragments of tetrandrine. ^{3}H-tetrandrine was successfully with a radiochemical purity of 90%.

KEY WORDS: d-tetrandrine, tritium gas exposure, hydroxide.

INTRODUCTION

Recently, d-tetrandrine has been found to be an important anti-cancer agent when used in conjunction with radiation therapy.[2,3] Tang and Huang reported[4] that 80 μg/ml d-tetrandrine, when incubated with BEL-7402† cancer cells, after 24 hours caused complete breakdown of these cells and when compared to control group (Figures 1-4).

Figure 1. Control BEL-7402 cells. The cells grew by the wall which is monolayer.

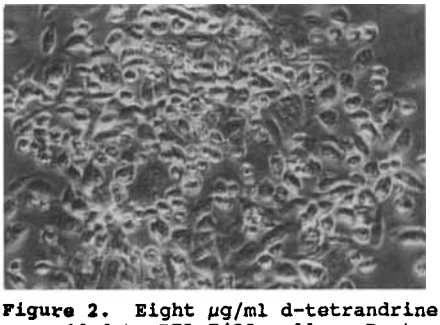

Figure 2. Eight μg/ml d-tetrandrine was added to BEL-7402 cells. Part of the cells were contracted.

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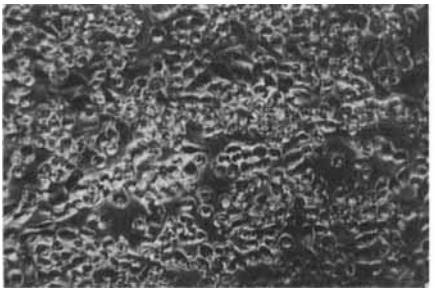


Figure 3. 24 μg/ml d-tetrandrine was added into BEL-7402 cells after 24 hours.

Most of cells were contracted float and aggregation.

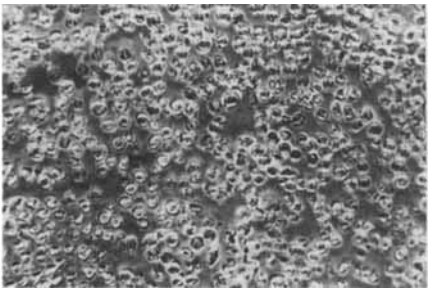


Figure 4. 80 μg/ml d-tetrandrine was added into BEL-7402 cells after 24 hours.

All of the cells were broken down.

Hui Fang reported[5] that in animals d-tetrandrine combined with a small dose of ^{60}CO showed anti-cancer effect.

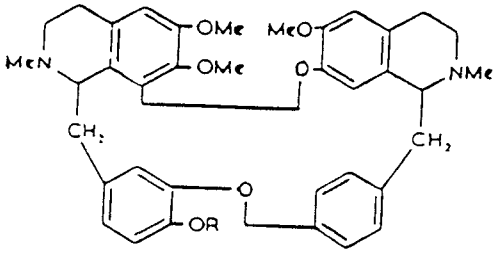
Dr. Gao Ling Shan et al.[6] reported 97 cases of stage II-VI lung cancer treated with d-tetrandrine combined with 2000 rad or 3000 rad ^{60}CO showed an obvious effect in 31.95%, a general effect in 29.8% and a stable effect in 28.8% by international standards. In order to investigate its pharmacology, the preparation of ^{3}H-tetrandrine is important because pharmacokinetics, bioavailability and tissue and organ distribution may be monitored by use of radioactively labeled ^{3}H-tetrandrine.

This report summarizes the preparation and isolation of ^{3}H-tetrandrine using tritium gas exposure. We have developed a new procedure for preparing high specific activity tritium labeled ^{3}H-tetrandrine. Such high specific activity may allow detection of even low levels of d-tetrandrine in infants and tissue, thus furthering pharmacological research and in turn, giving direction to clinical therapy.

RESULTS AND DISCUSSIONS

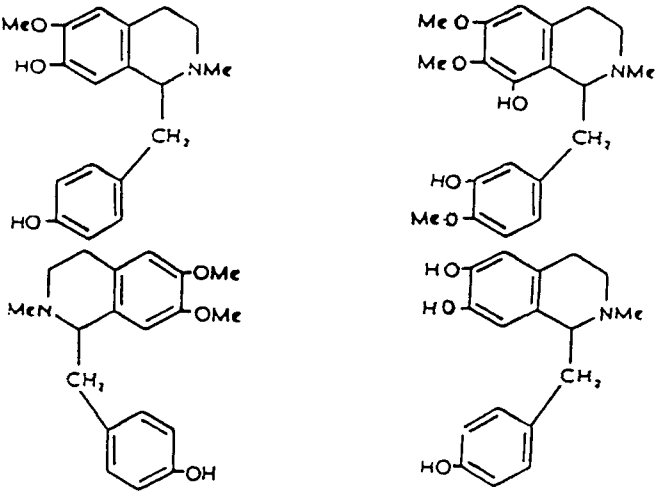
As seen in figure 5, d-tetrandrine is a complex structure.

Figure 5



When unlabeled d-tetrandrine was refluence with HBr, it broke down into many fragments. We collected these fragments from a column chromatograph and determined their structure by nmr and infrared spectrum. The structure of most of the fragments is seen in figure 6.

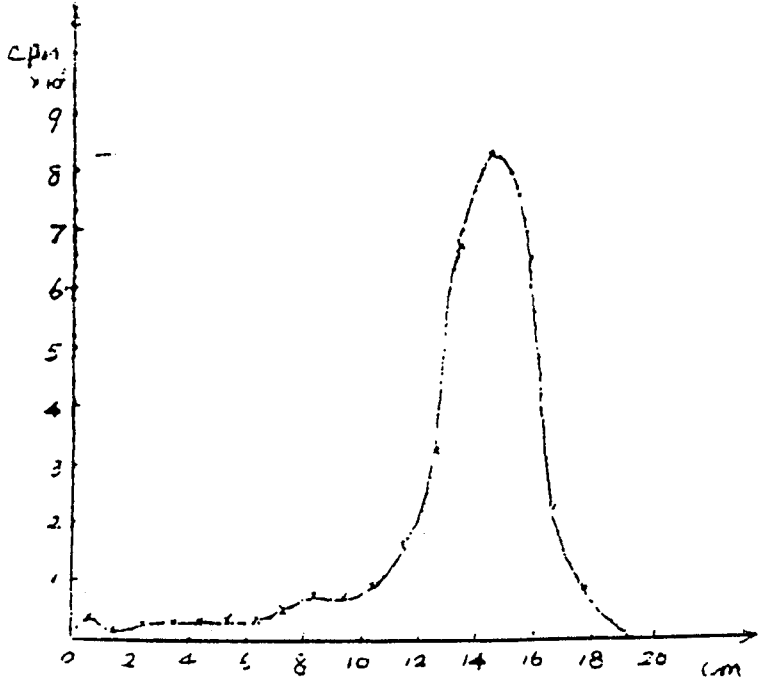
Figure 6



Almost all of these fragments contain hydroxide. Using this information, we developed a new procedure for the purification of ^{3}H-tetrandrine. A pH 4.7 Na_2HPO_4-citric acid buffer was smeared over a 2-3 cm zone, 3-5 cm in front of the original point on the paper. All hydroxide fragments of d-tetrandrine were blocked at the buffer location while the ^{3}H-tetrandrine could pass through this buffer zone in chloroform solvent. This new procedure produced ^{3}H-tetrandrine with a high specific activity 35 mci/mM and a radio-chemical purity >90%. As seen in figure 7, one peak.

Figure 7

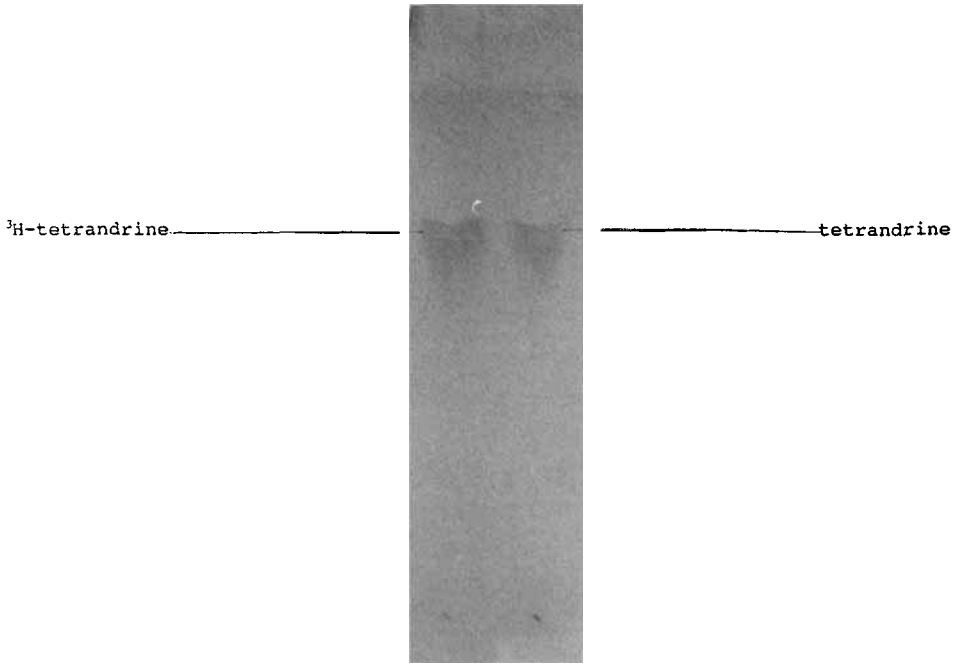
Detection of ^{3}H-tetrandrine



Radiochemical purity 90%

^{3}H-tetrandrine was identified by paper chromatography. As only one spot with a RF = 0.68 which is similar to standard d-tetrandrine Rf = 0.69 as seen in Figure 8.

Figure 8



EXPERIMENT

1. Identifying of ^{3}H-tetrandrine and radiochemical purity measurements were determined by the following methods:

   a. Quantitative measurements of the concentration and identifying of ^{3}H-tetrandrine were performed by paper chromatography on Xin-Hua #3 filter paper. The developing solvent system was Butanol:acetic acid:water (4:1:5). Color development reagent was Dregendoff reagent. The pure unlabeled standard d-tetrandrine was used as a control.

   b. Radioactivity measurements were made in a WE-8312 liquid scintillation spectrometer.

2. The d-tetrandrine was extracted from roots of Stephania tetrandrine S. Moore:Stephania tetrandra roots 3 kg were collected from the mountains of Jin-Hua P.R. China. The roots were cleaned with water and sunned to dry. They were crushed to powder and immersed in 2000 ml of a 2% HCl solution for 3 days. This solution was filtered and adjusted to pH 8 with 20% NaOH. 2000 ml of chloroform was added 5 times to extract d-tetrandrine. The extraction was concentrated by distillation to a brown crude of 20g. 2% HCl was added to dissolve, then filtered to remove undissolved material. This solution was adjusted to pH 8 with 10% NaOH. Benzene was added to remove hydroxide-tetrandrine; chloroform was added to extract 3-5 times. The above chloroform layer was concentrated to 10 c.c. to go on a column chromatograph. The products were recrystallized with acetone to yield needle crystal with an mp of 217-218 $C_{38}H_{42}N_2O_6$ (C 73.25; H 6.81; N.4.52; O 15.43). Thin layer chromatography (TLC) showed a spot. Its structure was determined by N.M.R. and infrared to be d-tetrandrine.

3. Labeling with tritium gas exposure.

The d-tetrandrine was dissolved in a small amount of chloroform, then injected into the exposure tube which was filled with glass fiber. Then the chloroform was removed by vacuum which left the d-tetrandrine distributed on the glass fibre. The exposure tube was connected to a high vacuum system, filled with 8 ci of tritium, then sealed. The d-tetrandrine powder was kept in the exposure tube for 18 days. The tube was then opened and the residue of tritium removed by vacuum. The d-tetrandrine color was changed from white to brown. To the above sample was added 2% HCl three times to dissolve and transferred to a small beaker. 2% NaOH was added for deactivation and neutralization , the precipitates of d-tetrandrine were filtered and the brown solution was discarded. The white precipitate was dissolved again in 2% HCl then neutralized with 2% NaOH. The d-tetrandrine was precipitated again. This procedure was repeated three times. The final precipitate is placed in chloroform to dissolve. If some brown precipitate still remains, it was filtered to remove. This solution was then paper chromatographed.

4. Paper chromatography:

The multiple buffer chromatography was performed as follows:

i. First step: A 2-3 cm with pH 4.7 Na_2HPO_4 - citric acid buffer zone was smeared 3-5 cm above the original point of a 20 cm x 30 cm Xin-Hua #3 filter paper as a block zone. The labeling tetrandrine chloroform solution was pointed on the filter paper and chloroform was used as a developing solvent. The chromatography was performed from bottom to top. The paper above the buffer zone was cut with scissors since it now contained the ^{3}H-tetrandrine. This paper was then rinsed with chloroform.

ii. Second step: The rinsed solution of chloroform was pointed on a 20 x 30 cm Xin-Hua #3 filter paper that had previously been immersed in 2% HCl. Butanol: acetic acid: H_2O (4:1:5) was used as developing solvent. The chromatography was developed from bottom to top. After completion, a length-wise strip of the paper was cut and sprayed with Dregendoff reagent, which produced a brown color. The corresponding location on the unsprayed paper was cut and raised with 2% Hcl. The pH of this solution was adjusted to 5-6 with 2% NaOH. This ^{3}H-tetrandrine was sealed in an ampoule and sterilized by high pressure for storage.

Acknowledgements:

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