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Study on the Correlation between the Structure and Toxicity of Amines, Mercaptans and Halohydrocarbons

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Abstract

The aim of the present work was to understand the structure of amines, mercaptans and halohydrocarbons and their cell toxicity effect on rat liver BRL-3A cells. BRL-3A cells were seeded into each well of 96-well plates and treated with amines, mercaptans and halohydrocarbons (total 18 kinds) in DMEM culture medium containing 10% fetal calf serum. 24h later, the growth viability in vitro of BRL3A cells was measured using the MTT assay. The results show that the cytotoxic of propane diamine> ethidene diamine> n-butylamine> n-propylamine> ethylamine, butyl mercaptan> propanethiol> disulfide propane> dithioglycol, n-Butyl iodide> n-Propyl iodide> iodoethane, propylene bromide> 1, 2-dibromoethane> ethyl bromide, ethylene dichloride> butyl chloride> chloropropane. We can conclude that among amines, when the length of carbon chain is fixed, the more amidogen, the stronger the toxicity; when the number of hydroxyl group is fixed, the longer carbon chain, the stronger the toxicity. Among mercaptans, when the length of carbon chain is the same, the more hydrosulphonyl, the weaker the toxicity; when the number of hydrosulphonyl is the same, the longer carbon chain, the weaker the toxicity. Among halohydrocarbons, when the length of carbon chain is fixed, the more halogen, the stronger the toxicity; when the number of halogen is fixed, the longer carbon chain, the stronger the toxicity. Under the same conditions, the toxicity of mercaptans is greater than amines and halohydrocarbons.

Keywords: The amines, The mercaptans, The halohydrocarbons, Rat liver BRL-3A cells, Median lethal dose (LD50)

Contents

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1. Introduction

Amines can be regarded as the ammonia derivatives whose hydrogen atoms are substituted by hydrocarbyl. In organic chemistry, the mercaptans are the non-aromatic compounds containing mercapto groups (-SH), or the mercaptans can be viewed as alcohols whose oxygen is replaced by sulfur. Halohydrocarbons refer to hydrocarbons whose hydrogen atom is replaced by halogen element, including fluorine (F), chlorine (Cl), bromine (Br), iodine (I), and astatine (At).

In daily life, amines, mercaptans and halohydrocarbons are prevalent and closely related to the environment, life, survival and health of human [1]. For example, amines are widespread in the biosphere, involved in many important physiological and biological activities, such as proteins, nucleic acids, many hormones, antibiotics and alkaloids are amines, most drugs used in clinical practice are also amines or amine derivatives [2].

Methyl mercaptan (MM, CH3SH) is one of the volatile sulfur compounds (VSCs), which are known to be involved in halitosis (bad breath) [3, 4] and periodontal diseases [4], and it is also the predominant causative factor of noticeable oral malodor [5]. Thus mastering their properties and synthesis methods is conducive to study the complicated natural products and better safeguard the human health.

In order to understand the structure of the amines, mercaptans, halohydrocarbons and their cell toxicity effect on rat liver BRL-3A cells.

The growth viability in vitro of BRL-3A cells was measured using the MTT assay and the results show that among amines, the toxicity increases with the increase of the amidogen when the length of carbon chain is fixed, and toxicity increases with the length of carbon chain increasing when the number of amidogen is fixed; For mercaptans, the toxicity decreases with the increase of the hydrosulphonyl when the length of carbon chain is fixed, and toxicity decreases with the length of carbon chain increasing when the number of hydrosulphonyl is fixed; For halohydrocarbons, the toxicity increases with the increase of the halogen when the length of carbon chain is fixed, and toxicity increases with the length of carbon chain increasing when the number of halogen is fixed.

2. Materials and Methods

2.1. Cell Culture

Rat liver BRL-3A cells were obtained from cell bank of the School of Basic Medicine of Peking Union Medical College (China). Cells were maintained at 37℃ in a humidified incubator containing 5% CO_2 and cultured in Dulbecco's modified Eagle's medium (DMEM, Life technologies, USA) supplemented with antibiotics (100U/ml penicillin and streptomycin) and 10% fetal bovine serum (Gibco, NY, USA) [6].

2.2. The Amines, Mercaptans and Halohydrocarbons

The compounds of this assay are analytical pure. The amines include propane diamine, ethidene diamine, n-butylamine, n-propylamine and ethylamine. The mercaptans include butyl mercaptan, propanethiol, disulfide propane and dithioglycol. The halohydrocarbons include n-Butyl iodide, n-Propyl iodide, iodoethane, propylene bromide, 1, 2-dibromoethane, ethyl bromide, ethylene dichloride, butyl chloride, chloropropane.

2.3. Drug Treatment of BRL-3A Cells

The cells in the logarithmic growth phase were dispersed with 0.25% trypsin, collected and diluted them to 2×10^4/ml with medium containing the 10% fetal bovine serum (Hangzhou Evergreen). Then seeded 100 μL above medium in 96-well culture plate per a hole, placed the plate in Heraeus sepatech at the condition of 37 ℃, saturation humidity, 5% CO_2 for 24 h. After that added the drugs (concentration including 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} mol/L) and then detected cell viability and apoptosis at 24h.

2.4. MTT Assay

The growth viability in vitro of BRL-3A cells was measured using the MTT assay [7, 8]. On the day of post-treatment 24h, 100 μl of spent medium was replaced with an equal volume of fresh medium containing MTT (Sigma) 0.5 mg/ml. Plates were incubated at 37°C for 4h, then the medium was replaced by 100 μl of DMSO (Sigma) and plates shaken at room temperature for 10 min. The absorbance was measured at 490 nm by Biotek reader (ELx800, USA). Three independent experiments were performed in three duplicates. In our study, the Inhibition Rate(IR)=(the control values-the experimental values)/ the control values×100%. Then the median lethal dose (LD50) was calculated by linear regression.

3. Results

3.1. The Amines' Cell Toxicity Effect on Rat BRL-3A Cells

The BRL-3A cells were treated by ethylamine, n-propylamine, n-butylamine, ethidene diamine and propane diamine respectively. The results showed that the median lethal dose of above drugs at 24h (LD50) was 18.1, 17.2, 5.5, 3.6 and 3.2 uM/L, indicating that the cell toxicity of propane diamine> ethidene diamine> n-butylamine> n-propylamine> ethylamine.

Table-1. amines' cell toxicity effect on rat BRL-3A cells

Names	MW(g/M)	Formula	Chemical formula	LD50(uM/L)
ethylamine	45	C2H7N	CH3-CH2-NH2	18.1
n-propylamine	59	C3H9N	CH3-CH2-CH2-NH2	17.2
n-butylamine	73	C4H11N	CH3-CH2-CH2-CH2-NH2	5.5
ethidene diamine	60	C2H8N2	H2N-CH2-CH2-NH2	3.6
propane diamine	74	C3H10N2	H2N-CH2-CH(NH2)-CH3	3.2

MW" stands for molecular weight, "LD50" stands for median lethal dose.

3.2. The Mercaptans' Cell Toxicity Effect on Rat BRL-3A Cells

The BRL-3A cells were treated by propanethiol, butyl mercaptan, dithioglycol and disulfide propane respectively. The results showed that the median lethal dose of above drugs at 24h (LD50) was 46.1, 58.6, 74.5 and 83.4 uM/L, indicating that the cell toxicity of butyl mercaptan> propanethiol> disulfide propane> dithioglycol.

Table-2. mercaptans' cell toxicity effect on rat BRL-3A cells

Names	MW(g/M)	Formula	Chemical formula	LD50(uM/L)
propanethiol	76	C3H8S	CH3-CH2-CH2-HS	46.1
butyl mercaptan	90	C4H10S	CH3-CH2-CH2-CH2-HS	58.6
dithioglycol	94	C2H4(SH)2	HS-CH2-CH2-HS	74.5
disulfide propane	108	C3H8S2	HS-CH2-CH2-CH2-HS	83.4

"MW" stands for molecular weight, "LD50" stands for median lethal dose.

3.3. The Halohydrocarbons' Cell Toxicity Effect on Rat BRL-3A Cells

The BRL-3A cells were treated by ethylene dichloride, butyl chloride, chloropropane, propylene bromide, 1,2-dibromoethane, ethyl bromide, n-Butyl iodide, n-Propyl iodide and iodoethane respectively. The results showed that the median lethal dose of above drugs at 24h (LD50) was 465.1, 597.5, 673.9, 318.5, 398.1, 3148.1, 121.2, 121.0 and 577.7 uM/L, indicating that the cell toxicity of ethylene dichloride> butyl chloride> chloropropane, propylene bromide> 1,2-dibromoethane> ethyl bromide, n-Butyl iodide> n-Propyl iodide> iodoethane.

Table-3. The halohydrocarbons' cell toxicity effect on rat BRL-3A cells

Names	MW(g/M)	Formula	Chemical formula	LD50(uM/L)
ethylene dichloride	143.5	C2H4Cl2	Cl(CH2)2Cl	465.1
butyl chloride	92.5	C4H9Cl	CH3CH2CH2CH2Cl	597.5
chloropropane	78.5	C3H7Cl	CH3-CH2-CH2-Cl	673.9
propylene bromide	202	C3H6Br2	CH3-CH(Br)-CH2-Br	318.5
1,2-dibromoethane	188	C2H4Br2	Br(CH2)2Br	398.1
ethyl bromide	109	C2H5Br	CH3-CH2-Br	3148.1
n-Butyl iodide	184	C4H9I	CH3-CH2-CH2-CH2-I	121.2
n-Propyl iodide	170	C3H7I	CH3-CH2-CH2-I	121.0
iodoethane	156	C2H5I	CH3CH2I	577.7

"MW" stands for molecular weight, "LD50" stands for median lethal dose.

3.4. Comparison Analysis of the Cell Toxicity Effect on Rat BRL-3A Cells between Amines, Mercaptans and Halohydrocarbons

When the rate of cell death is 50%, the concentration of the n-butylamine, butyl mercaptan, n-Butyl iodide and butyl chloride is 5.5, 0.8, 121.3 and 597.5 uM/L respectively (Fig.1A). The concentration of n-propylamine, propanethiol, n-Propyl iodide and chloropropane is 17.2, 0.8, 121.0 and 673.9 uM/L respectively (Fig.1B). The concentration of ethylamine, iodoethane and ethyl bromide is 18.1, 577.7 and 3148.1 uM/L respectively (Fig.1C). The concentration of ethidene diamine, dithioglycol, 1, 2-dibromoethane and ethylene dichloride is 3.6, 1.1, 398.1 and 465.1 uM/L respectively (Fig.1D). These results indicate that when the length of the carbon chain is the same, the toxicity of mercaptans is greater than amines and halohydrocarbons.

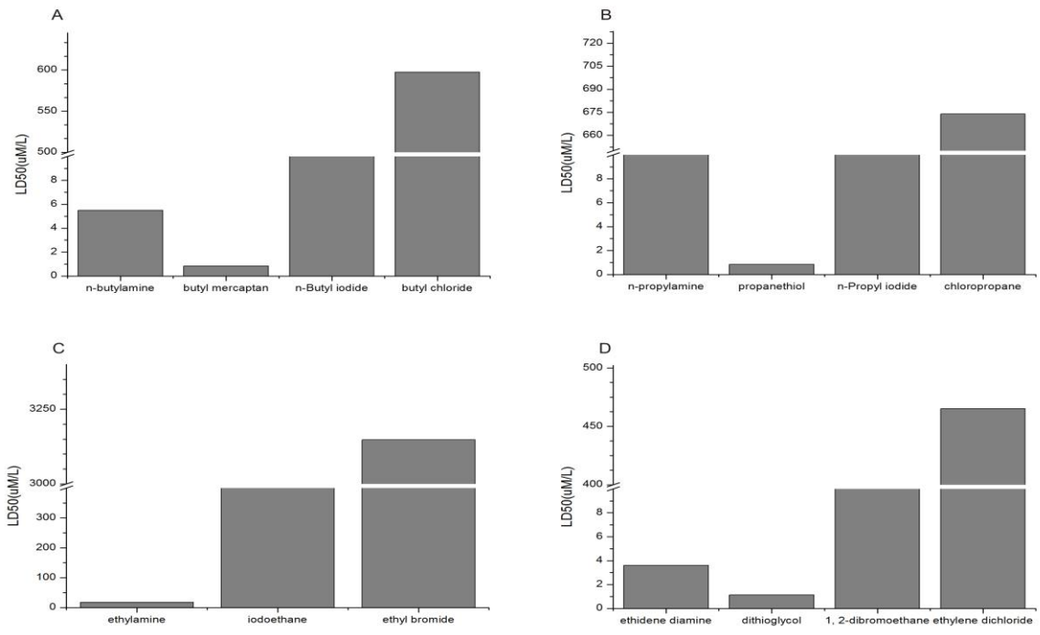

Figure-1. Comparison analysis of the cell toxicity effect on rat BRL-3A cells between amines, mercaptans and halohydrocarbons

The break region are (A) from 10~600uM/L, (B) from 10~650uM/L, (C) from 400~3000uM/L, (D) from 10~400uM/L

4. Discussion

Studies have shown that amines are alkaline and easily reacted with the acidic group of nucleic acids and proteins, and also easily reacted with enzyme. In the present study, more amino groups demonstrate stronger cell toxicity; when the number of amino groups is fixed, more carbon atoms indicate higher virulence. This may because that the not-shared electron pair of the nitrogen atom can form the hydrogen bond, which can strongly interfere with the amine metabolism. On the other hand, more amino groups demonstrate stronger cell toxicity, which is due to the increasing solubility of amines caused by the increase of hydrogen bonds [9]. Besides, when the number of amino is fixed, more carbon atoms may increase the lipid solubility and the cytotoxicity is also enhanced.

Studies have shown that there is a dipole attraction between mercaptans, and there is neither a significant hydrogen bonding interaction nor association between them. But it is easy to generate a variety of mercaptides when reaction with metal ions, and to conduct addition reactions with the compounds containing double bonds, suggesting its high chemical activity. In the present study, more mercapto groups demonstrate weaker cell toxicity; when the number of mercapto groups is fixed, more carbon atoms indicate lower virulence. This maybe because mercaptans can not form hydrogen bonds with water, which led to the decrease of its solubility in water, and the decreasing of toxicity also caused by the increase of molecular weight [10].

Since halogen elements have a strong electron-withdrawing effect, the molecular polarity would increase when halogen is introduced to molecular. Then the molecular can easily combine with enzyme system of cells, which may lead to abnormal metabolism in cells and organism. When the number of halogen elements is fixed, more carbon atoms indicate higher virulence, which is due to the increasing solubility of halohydrocarbons caused by the increase of hydrocarbyls. In addition, as the strength of electron-withdrawing effect is I＞Br＞Cl＞F [11], the toxicity of iodohydrocarbon＞bromohydrocarbon＞chlorohydrocarbon＞ fluorohydrocarbon.

In conclusion, these results provide more information for further analysis of their relationship between structure and cell toxicity, the mechanism in vivo and establishing measures to prevent their toxic effects.

5. Conflict of Interest

The authors confirm that this article content has no conflicts of interest.

6. Acknowledgement

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References

[1] Z. H. Li, S. G. Sun, and J. L. Marty, "Design and characterization of methyl mercaptan biosensor using alcohol oxidase," *Sensors and Actuators B*, vol. 192, pp. 680-684, 2014.

[2] J. Murphy, R. Everley, J. Coloff, and S. Gygi, "Combining amine metabolomics and quantitative proteomics of cancer cells using derivatization with isobaric tags," *Anal. Chem.*, vol. 86, pp. 3585-3593, 2014.

[3] J. Tonzetich and P. A. Carpenter, "Production of volatile sulphur compounds from cysteine, cystine and methionine by human dental plague," *Arch. Oral Biol.*, vol. 16, pp. 599-607, 1971.

[4] J. Tonzetich, "Production and origin of oral malodor: A review of mechanisms and methods of analysis," *J. Periodontol*, vol. 48, pp. 13-20, 1977.

[5] S. Awano, S. Koshimune, E. Kurihara, K. Gohara, A. Sakai, I. Soh, T. Hamasaki, T. Ansai, and T. Takehara, "The assessment of methyl mercaptan, an important clinical marker for the diagnosis of oral malodor," *J. Dent.*, vol. 32, pp. 555-559, 2004.

[6] R. C. Dokko, B. H. S. Cho, and B. H. Chung, "Cellular uptake of stearic, oleic, linoleic, and linolenic acid and their effects on synthesis and secretion of lipids in Hep-G2 cells," *Int. J. Biochem. Cell. Biol.*, vol. 30, pp. 65-76, 1998.

[7] K. Xiang-Ping, Z. Guo-Chi, and Z. Yi-Jun, "Experimental study on the activity of hepatocyte stimulating factor," *Tianjin Medical Jounal*, vol. 17, pp. 401-404, 1989.

[8] Q. Cai-Rong, L. Xin-Tian, and L. Chao-Liang, "Determination of the biological activity of HGF on the growth of SMMC-7721 cells by MTT assay [J]," *Chinese Journal of Hospital Pharmacy*, vol. 26, pp. 42-44, 2006.

[9] L. Kromer, A. Coelho, I. Bento, A. Marques, and C. Romão, "The effect of specific modifications of the amine ligands on the solubility, stability, CO release to myoglobin and whole blood, cell toxicity and haemolytic index of [Mo(CO)4(NR3)2] complexes," *Journal of Organometallic Chemistry*, vol. 760, pp. 89-100, 2014.

[10] B. H. Simon Cho and X. Shanqin, "Effects of allyl mercaptan and various allium-derived compounds on cholesterol synthesis and secretion in Hep-G2 cells," *Comparative Biochemistry and Physiology Part C*, vol. 126, pp. 195-20, 2000.

[11] J. Holder, "Analysis of chloroethane toxicity and carcinogenicity including a comparison with bromoethane," *Toxicology and Industrial Health*, vol. 24, pp. 655-675, 2008.