ICMS 2008

The International Crisis Management Symposium
on CBRN and Emerging Infectious Diseases

September 13th - 16th, 2008

Chiba Institute of Science (Choshi, Japan)

Proceeding

(Digital Version)

Organizing Committee of CIS Symposium 2008

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Risk management for radiation

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Abstract

Radiation gives us great useful tools for the cancer treatments by radiotherapy and supporting our energy consumption by the massive energy release from nuclear reaction in power plants. On the other hands, radiation is known as double edged swords. Biologically, ionizing radiation releases local energy deposition and produces ionizing water and highly reactive radicals in our body. It leads to break DNA strands and it leads to cell death, mutation, and carcinogenesis. To prevent the worst scenarios, we have to know well about the characters of radiation and its history. We will discuss here about radiation accidents in Japan and world and radiological decontamination. Then, we will explain the background level radiation exposure and a possible increased cancer cases in Japan by a high usage of medical diagnostic x-ray. Finally radiological casualties and classic and new biomarkers for radiation dosimeters will be discussed.

Basic Radiation Biology

Radiobiology is the study of the action of ionizing radiations on living things. The absorption of energy from radiation in biologic materials may lead to excitation or to ionization locally. Like X-ray, radio waves, radar, radiant heat, and visible light are forms of electromagnetic radiation. They have same velocity, $c=3x10^9$m/s, but they have different wavelengths and frequencies. X-ray and gamma-ray produced extranuclearly or intranuclearly have short frequency and can ionize molecules directly. And neutron, alpha particles, beta-ray, heavy charged particles are also known as particle ionizing radiation. The first step in their absorption is the production of fast recoil electrons. After that, the atoms of target molecules may be ionized directly or indirectly by high reactive free radicals. Since ionizing radiation locally produces ionized free radicals, it easily breaks DNA strands. It is the unique character of ionizing radiation. Broken DNA can be repaired, but un-repaired or mis-repaired broken DNA caused cell death, mutation, and carcinogenesis (1).

Key words: radiation, decontamination, background radiation, acute high dose radiation

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Table 1; Scheme of radiation events.

10^{-15} seconds	Radiation Event
10^{-3} ~10^{-5} seconds	Radical Formation
Minutes Hours	DNA Breaks and Repair
Days	Cell Loss
Weeks Months	Death
Years	Carcinogenesis

Different of doses of radiation will give different modes of death to man. 10Gy of radiation causes gastrointestinal death within 9 days. 3-8 Gy of exposure leads to hematopoietic death about 3 weeks or more. The $LD_{50/60}$ (the dose for half of human death within 60days) for human is 3 to 4 Gy for young adults without medical intervention. The duration time between exposure and death are associated with DNA repair and cell division. The loss of fast growing gastrointestinal cells results in the loss of body fluid from intestine. Radiation damages and kills stem cells and it prevents organs from producing renewal mature functioning cells. In fact, a bone-marrow transplant is effective in small window between 8-10 Gy radiation exposures (1).

Table 2; Acute radiation effects

0.25-1Gy	Subclinical range, minor blood chemistry changes
1-2Gy	White blood cell loss
>2.5Gy	Acute radiation syndrome
>3.5Gy	Hematopoetic syndrome
4.5Gy	LD50/60
>6Gy	Gastrointestinal syndrome
10Gy	LD100/60
>10Gy	Central nervous system syndrome

Not only cell killing, radiation has effect for mutagenesis and carcinogenesis. Radiation carcinogenesis is a stochastic effect, the probability of an effect increases with dose, with no dose threshold. Cataracts are an example of a deterministic effect of radiation. After atomic bombing in Hiroshima and Nagasaki, about 120,000 persons have been followed cancer incidence including about 50,000 received doses in excess of 5mSv. By 1990, there had been 6,000 deaths from cancer of which about 400 were considered to be an excess mortality caused by radiation. The latency of tumorgenesis is dependent on tumors. Leukemia has the shorted latent period (7-12 years), and solid tumors showed a long latency than the leukemia (10-50 years) from the survivors of Hiroshima and Nagasaki.

Radiation Accidents

So many radiation accidents have been reported not only Japan and the world. Some of them extremely damaged and contaminated to human and nature. Others did not as expected. Accidents associated radiation and radioactive materials can be separated into two category open type and closed type. The open type radiation accident is actual release of radionucleotide into nature. In Chernobyl power plant accidents, huge amounts of radionuclides were released to open space and spread onto Europe. The amounts of radioisotope is estimated about 10t including about $1500x10^{15}$Bq of ^{131}I and $80x10^{15}$Bq of ^{137}Cs. Although still 10km radius from power plant is limited to access, there is a real wild life protection area around Chernobyl and many wild lives increases their number. A critical accident occurred in 1999 at a uranium conversion facility in Tokaimura, Ibaraki, Japan. The criticality was unexpectedly initiated when a worker on the platform was leaning over a precipitation tank, pouring a solution of uranyl nitrate enriched in ^{235}U from a stainless steel bucket in the tank through a funnel. Of course, this critical accidents release many radionuclides from the facility like Chernobyl. Only those workers were exposed to significant amount of radiation. Three workers developed acute radiation syndrome and they were transferred to the National Mito Hospital once. And then the patients were transferred to National Institute of Radiological Sciences in Chiba 5 hours after exposure by helicopter to further evaluation and treatment. Based on several methods of dose estimation, three workers were exposed to 16-20 Gy equivalent, 6-10 Gy equivalent, and 1 to 4.5 Gy equivalent.

Table3; Estimated doses in Gray for three workers in Tokai

	Worker A	Worker B	Worker C
Neutron	5.5	2.9	0.81
Gamma-ray	8.5	4.5	1.3

Worker A and B were transferred to the University of Tokyo Hospital for intensive higher care. Worker A died of multiple organ failure on Day 82. And Worker B succumbed to refractory respiration failure on Day 211 (2).

Compared with direct exposure from radiation accidents as mentioned above, indirect exposure from radiation fall out or radiological contamination can be reduced the amount of exposure by radiological decontamination. The primary differences between the mechanics of radiological decontamination and chemical decontamination are the methods of monitoring and timing. Chemical decontamination is an emergency.

Decontamination of casualties is an enormous task. The process requires dedication of both large numbers of personnel and large amount of time with appropriate training and planning. Removal of outer clothing and washing exposed skin and hair removes 95% of contamination.

Radiation Exposure in Medicine

Everyone is exposed to radiation from unperturbed natural sources (cosmic rays, terrestrial radiation, and natural compounds of our body), enhanced natural sources (airplane travel, radon exposure), and sources from human activity such as nuclear medicine. Intensity of cosmic rays arriving

at the earth's surface varies with both latitude and altitude above sea level. Average equivalent dose in the USA is about 0.26mSv/year, but in Denver, Colorado (a mile high city), it is 0.5mSv/year. The biggest source of natural background radiation is radon gas in the USA, which seeps into the basement of houses from rocks underground.

There are several inhabited areas of the world where background radiation is considerably high because of radioactivity in rocks or soil or building materials. In Brazil, about 30,000 people live in coastal areas are exposed to dose rate of 5mSv/yr (20 times higher). The highest background is in Kerala, India, where more than 100,000 people receive an annual dose of about 13mSv (50 times higher). After many researches have been made of these human populations, so far no excess incidence of caner or hereditary abnormalities have been reported by radiation.

In USA, annul effective dose was about 3.6mSv. 55% comes from radon gas. 18% is man made nuclear medicine. Internal was 11%, terrestrial was 8%, and cosmic was 8% in 1987. In Japan, annual effective dose is about 3.75mSv. About 60% comes from nuclear medicine, and contribution of radon is 10%. Nuclear medicine is not natural source of background radiation. One paper published to famous medial journal Lancet in 2004 showed that the usage of medical radiation diagnosis is three times higher in Japan than other western countries and it contributes cancer incidence in Japan. They estimated 3.2% of cancer patients in Japan were caused by x-ray diagnosis, however, it is still unclear and controversy (3).

Table 4; Annual natural background radiation exposures in USA and Japan.

	USA	Japan
Radon	2.0mSv	0.4 mSv
Nuclear Medicine, Diagnose	0.53 mSv	2.25 mSv
Others	1.07 mSv	1.1 mSv
Total	3.6 mSv	3.75 mSv

Table 5: Medical diagnostics and radiation exposure.

Medical Diagnostics	**mSv**
Chest x-ray	0.1
Dental oral exam	1.6
Mammogram	2.5
Lumbosacral spine	3.2
PET	3.7
Bone (Tc-99m)	4.4
Cardiac (Tc-99m)	7.5
Cranial CT (MSAD)	50
Barium contract G-I fluoroscopy (2min)	85
Spiral CT	30-100

Risks in Nuclear Affairs

Acute high dose radiation occurs in three principal situations. A nuclear weapon will result in extremely high dose rates from initial nuclear reaction and the fallout (Hiroshima and Nagasaki). Highly enriched nuclear material is allowed to form a critical mass. Then, nuclear reaction releases the large amount of neutrons and gamma-ray (Chernobyl and Tokai). A radiation dispersal device made form from highly radioactive materials (Dirty bomb).

Nuclear weapons cause not only acute radiation syndrome but also the blast and thermal biological effects. Blast causalities will require evaluation for acute trauma in accordance with advanced trauma life-support standard therapies. Thermal burns will be the most common injuries, subsequent to both the thermal pulse and the fires it ignites. We should not forget the anxiety by radiation stress. The severity of the psychological effects of a dirty bomb will depend on the nature of the material itself and the method of deployment. Although a point source such nuclear weapons targets physical damages and injuries, dirty bombs will produce more detrimental psychological damage to civilians.

Each case, measurement of actual doses for radiation exposure is important to understand the radiological damages and to help the medical and psychological cares. The classic dosimetry assays are based on several biomarkers. Onset of vomiting with in 12 hours indicates the radiation exposure from 2Gy to 20Gy. Depletion of peripheral blood lymphocytes within 10days suggests the doses from 2 to 8 Gy. Cytogenetics techniques, lymphocyte-metaphase spread dicentric and premature chromosome condensation assays can detect doses from 0.2 Gy to 20Gy. Prodromal signs and symptoms and hematological analysis can be quick but not accurate to determine the doses. Although these cytogenetic assays are so sensitive to determine the actual doses, sample blood have to be collected 24 hours after radiation exposure and need time and professional handling for analysis.

Several practical and easy assays are developing for new radiation exposure biomarkers for the high throughput analysis. Recent studies for γ H2AX foci can measure each DNA double strand breaks and estimate actual exposure and handling is easer than conventional cytogenetics. In this technique, isolated lymphocytes can be fixed and stained with antibody against γ H2AX. Actual measurements can be done with microscopic analysis or automated flow cytometer.

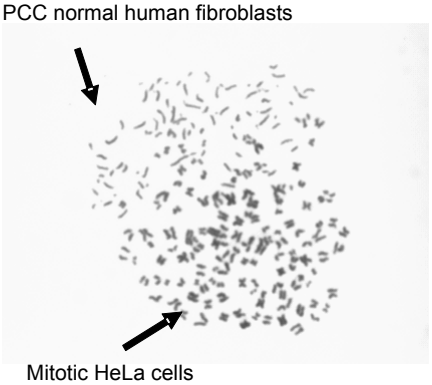

Figure 1; Premature Chromosome Condensation

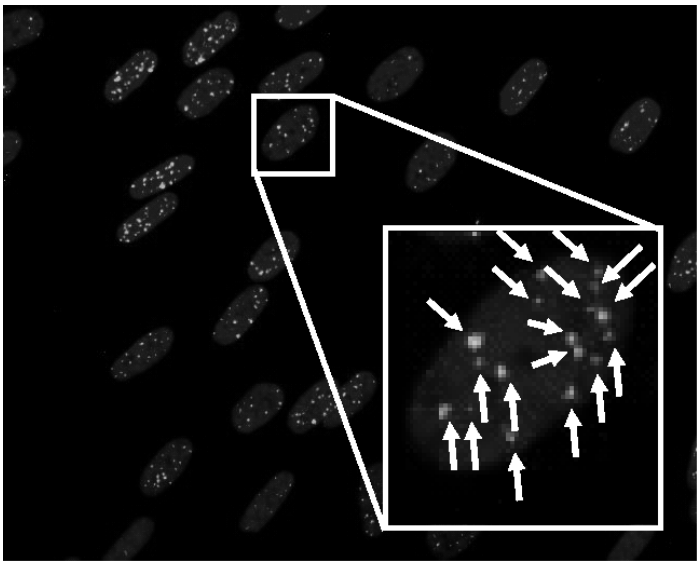

Figure2; γH2AX foci after 1Gy of irradiation.

Acknowledgments

We appreciate the Organizing Committee of CIS Symposium 2008 for giving us a chance to present our talks. We thank Dr. Anthony Tu and Dr. Hiroyasu Ohtaka for helping to organize the session.

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An Application of Computer Simulations for People Evacuation Management in a Complex Setting

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Abstract

Natural and man-made hazard does not necessarily result in disaster in an area. It hinges upon: (1) the risk and the strength of the hazard; (2) the population density; (3) the integrity of structures; and (4) the efficiency of hazard management implemented in the areas. From this perspective, Asia Pacific Region is one of the highly risky regions in view of the tremendously growth in the urbanization process. The super high density of population and the super high rise structures in this region pose an immense risk of disastrous consequence just even triggered by a trivial natural or man-made hazard. Recognizing this, a variety of organizations at all levels of government and in the public and private sectors have increased consideration of emergency management.

We understand that absolute prevention of disasters and restricting their spread may be impossible. Evacuation of people from the hazardous region(s) is *per se* a way to reduce the ill effects of disasters and evacuation planning is *prima facie* one of the critical components in emergency management. This article briefly outlines the framework of emergency management and discusses the use of computer simulations for evaluating different people's evacuation strategies for an urban area.

Introduction

Rapid urbanization and concentration of people and facilities in the metropolitan areas cause huge demand of floor space in many cities. Super high-rise and complex buildings and estates are now constructed everywhere, in particular in many metropolitan areas in the Far East. Of all the issues relating to such complex society, safety is one of the major concerns of many people as well as the government. In the circumstance, societies have attempted to establish systems to 'manage' the natural and technological hazards and their impacts on life and property.

It is well-known that absolute elimination of hazard appears impossible. Nevertheless, we should endeavor to minimize the possibility of happening of hazardous events. If unfortunately such an event happens, the system should be able to limit its effect such as in case of a fire incident, controlling the spread of the fire and smoke. Failure to control the spread of the hazards, people in the area should be evacuated immediately, and emergency personnel should be able to gain access to the site to assist the evacuation and perform rescue.

Keywords: Emergency Management, Evacuation, Simulation

This article begins by providing a brief outline of emergency management. A review of the importance of evacuation is then discussed. Particular introduction will be given to the use of a tool – computer simulation for analyzing the pattern of people's movement efficiency of the crowd flow process in evacuation.

Emergency Planning

Natural and man-made hazard does not necessarily result in disaster in an area. It hinges upon: (1) the risk and the strength of the hazard; (2) the population density; (3) the integrity of structures; and (4) the efficiency of hazard management implemented in the areas. From this perspective, the super high density of population and the super high rise structures in the Far East region pose an immense risk of disastrous consequence just even triggered by a trivial natural or man-made hazard. Recognizing this, a variety of organizations at all levels of government and in the public and private sectors have increased consideration of emergency planning and management. Cigler (1987) has described that emergency planning is the process of 'developing and implementing policies and programmes to avoid and cope with the risks to people and property from natural and man-made hazards'.

Managing emergency incidents effectively can be discussed and encapsulated in an emergency event continuum. Table 1 summarises the four stages that an emergency event (a disaster) may pass through.

Table 1: Outline stages in the emergency continuum

Emergency events continuum		**What involved?**	**What to do?**
Pre-event	Mitigation	• Identify hazard and assess the effect • Develop the approach to reduce vulnerability • Verify the scope of risks	• Plan the land use and control mechanism • Formulate building and fire codes • Initial studies relating to emergency management
	Preparedness	• Analyse the consequence of an event • Establish appropriate reaction mechanism, etc. • Identify inter-agencies roles • Determine detection mechanism, etc.	• Establish reaction strategies (including evacuation strategies) and detection system • Train key emergency personnel • Formulate inter-agency cooperation
Post-event	Response	• Establish warning system • Formulate communication structures • Designate emergency control point • Formulate the ways to control/ extinguish the hazardous event	• Initiate warning system • Notify emergency service • Establish control point • Perform control and rescue process • Maintain the functions, as far as practicable, of the community • Support officials empowered for action
	Recovery	• Establish restore programmes • Plan rehabilitation (including buildings, environment, etc) • Mobilise and review the communities resources	• Restore to normality as quickly as possible • Revitalise the destruction

In crisis situations, such as tsunami, nuclear, chemical, terrorist attack emergencies, appropriate decision making holds the core to alleviating the intensity of the ruinous impacts of the disaster. Often speed and accuracy are success determining factors for the decision making. It is therefore, crucial that planning and preparation processes for potential emergencies are carried out in advance. The mitigation and preparedness planning at the pre-event stage are significant. Figure 1 outlines a brief approach.

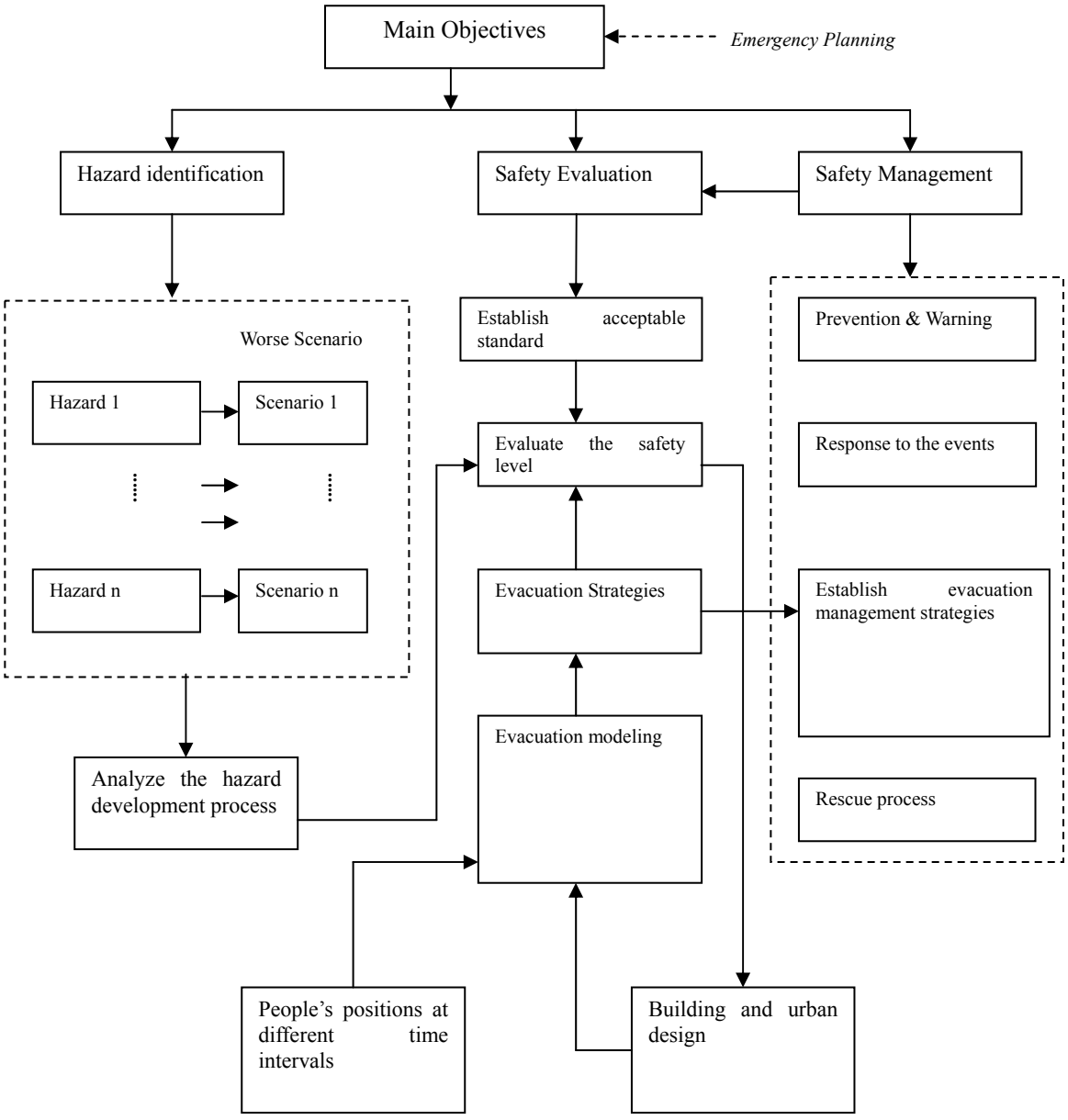

Figure 1: An outline of emergency planning

Evacuation

Evacuation of people from the hazardous region(s) is *per se* a way to reduce the ill effects of disasters and evacuation planning is *prima facie* a critical component in emergency management.

Moving ahead of the danger is the better practice. Some recent disasters caused by the 'attack' of hurricanes have demonstrated that timely evacuation should be a means of mitigation to the ill-effect. It is recognized that two factors have a major impact on evacuation strategies: the timing of evacuation relative to disaster impact, and the amount of time in which evacuees are required to leave the evacuation planning zone. The former component, which is not discussed in this article, requires the development of an effective hazard detection and warning system. Even if an effective detection system has been established, it is useless if the threatened areas cannot be effectively evacuated in the determined time. Thus, the latter issue that involves evacuation planning arouses the interest of many management scientists.

Evacuation planning may be categorized into simulation models approach and analytical models approach. Figure 2 briefly describes the major models adopted for evacuation planning.

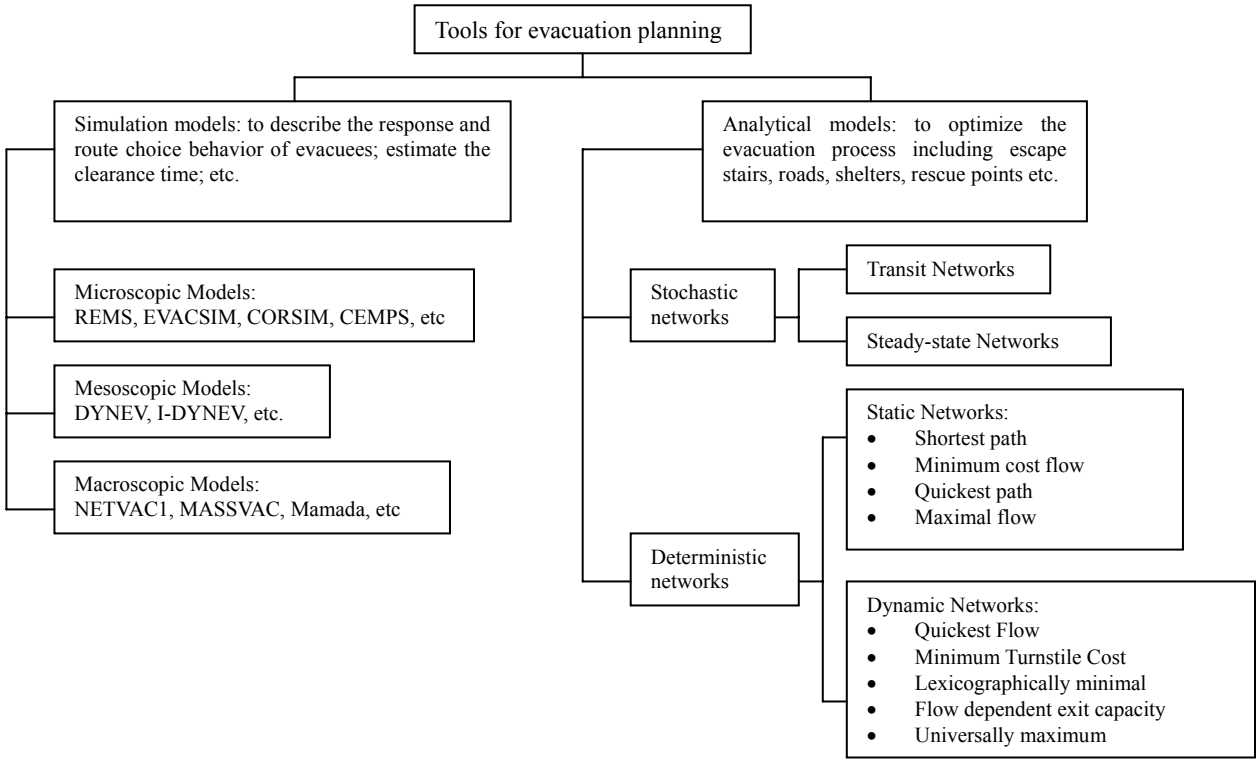

Fig. 2 Major Models for Evacuation Planning

Applications

An important aspect of ensuring safe evacuation in a building or a district is the availability of a sufficient number of adequately sized and safe escape routes for the people to leave the hazardous zone within an appropriate period of time. Normally the time being considered is the time before the zone becomes hazardous to the people. In general, building and fire codes and other planning guidelines provide guidance on the design of egress routes in terms of size and capacity. However, individual components conform to the guidelines, but as a collected whole, they may lack the coordination necessary for the inducement of smooth egress during evacuation, especially in a

complex setting with numerous people and traffic volume.

Apart from the space planning initiated by the building and urban designers, emergency management personnel may also need to understand the possible crowd and traffic flow pattern so that they can plan to control the flow pattern in order to ensure a smooth evacuation.

In order to facilitate the understanding of dynamic crowd flow pattern, researchers in recent years have developed many evacuation models. With the advancement of computer technology, some of the models are so sophisticated that each individual's movement in the concerned area can be traced and their movement path can be visualized, such as the EXODUS (Galea, 1994), SIMULEX (Thompson *et al*, 1995), EGRESS (Ketchell et al, 1993), SGEM (Lo *et al*, 2004), PEDGO (Klupfel, 2004), etc. The simulation process is adopted to build up some kind of artificial model or simplified representation which resembles the real world system.

Evacuation under emergency situation for a complex setting is too complex to be represented by simple flow equations and can hardly be initiated for experiment (fire drill exercise cannot be regarded as a real experiment). Therefore, the simulation model may be an alternative approach to anticipate in advance how the system will react should emergency situation arises. During the implementation stages, a simulation model can be used to experiment with new designs or policies, so as to prepare for what may happen. Valuable insight may be obtained by changing simulation inputs and observing the resulting output.

The following simulation outputs (Figure 3 - 5) are extracted from the model SGEM (Lo, *et al*, 2000, 2004, 2006; Zhi *et al*, 2003) developed by the author at the Department of Building and Construction, City University of Hong Kong. The model is basically a fine grid model which can adopt the spatial information from CAD based architectural plans to perform simulation to generate the crowd movement pattern. The model can provide reasonable results under large population situations. However, in case where only few people located in the area, the people's behavioral reaction will have a significant effect on the evacuation process.

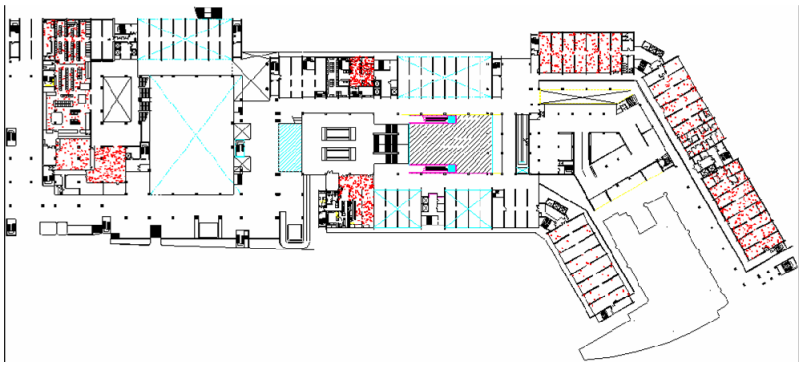

Figure 3: Simulation output of a large shopping mall

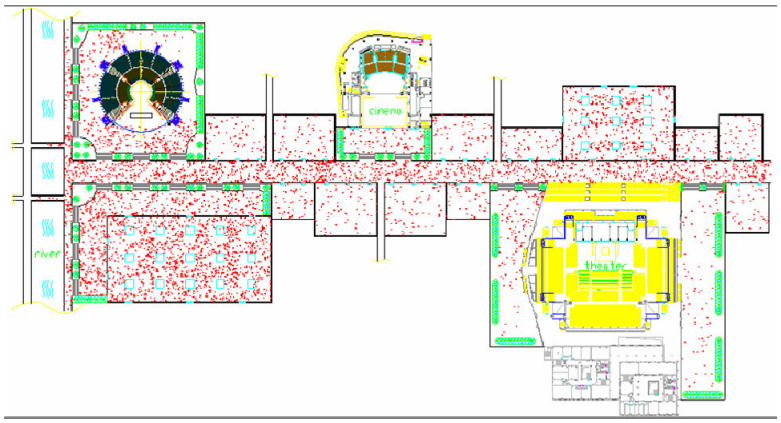

Figure 4: Simulation output of a pedestrian walkway

Figure 5(a): Simulation output of crowd flow in a mass transit station

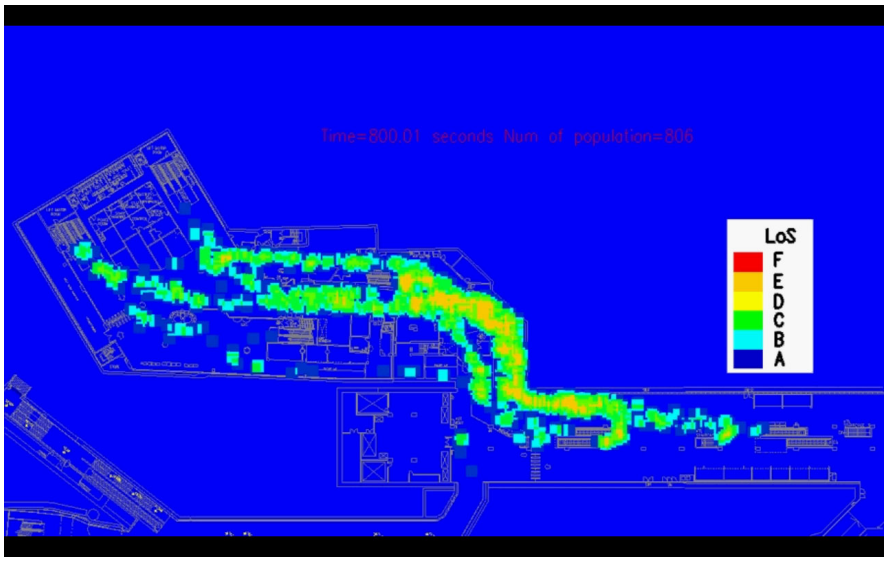

Figure 5(b): Simulation output of the crowd density in a mass transit station

The results given by the simulation outputs (Figure 4-5 refers) can be adopted to analyze the crowd flow pattern. Critical points may be obtained by tracking the dynamic crowd density. Fruin (1971) has pointed out that crowd may crush and endanger the people in the crowd if the density is about or over 5.6 person per square metre. The model can provide the crowd density information at various point of time. If it is greater than the acceptable value, which should be lower than that indicated by Fruin, for some time, the setting adjacent to the critical points should be altered. Control the amount of people flowing to the critical points may also be necessary.

Concluding Remarks

The article presents a brief outline of emergency planning and the use of computer simulation model for evacuation analysis. The simulation output can offer valuable information for evaluating the crowd flow pattern, which can serve as a reference for determining the effective layout at design stage and formulating the crowd management strategies.

Nevertheless, it should be pointed out that social science of emergency egress is also important. People's behavior is partly the result of what threatens them. Evacuations are a function of a system of interactions among characteristics of the hazards, characteristics of the social and cultural organization of an area in case of evacuation for a large district, characteristics of the escape routes and if for a large district, the road and transportation system, and characteristics of the warning and emergency management system.

Different disasters should have different detection systems and different warning modalities. The emergency management system of an area is also not uniform for all hazards, but will be more or less prepared to respond if not mitigate the effects of the various hazards causing the disaster as well as the effects of the disasters. Importantly, often hazards or disasters may block or even destroy the escape routes such as staircase, or road and transportation structures, which must be factored in as the simulation goes forward.

The evacuation simulation models cannot provide the insights for all the components in an emergency management system. An effective emergency management system should be established by considering all aspects including mitigation, preparedness, response and recovery.

Acknowledgments

The work described in this paper was fully supported by the grants from the Research Grant Council No. CityU118107.

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医療過誤防止と医薬品品質確保のための製剤設計・製造設計

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1．はじめに

医薬品等における「誤認」「誤薬」は，医療機関の規模に関係なく発生する可能性があるため，各医療機関は職員の啓発・教育のみならず，それぞれ独自の事故防止システムを積極的に導入している．一方，我々医薬品メーカー側においても，医薬品の「誤認」「誤薬」を可能な限り回避できるような製剤設計に努めるとともに，一方では，健康被害の原因ともなりうる製造工程における医薬品の汚染を防止するために，製造システムの設計とさらなる改良にたゆまぬ努力を傾注している．今回は，医薬品メーカーにおけるこれらの製剤設計，製造設計の事例を簡単に紹介する．

一般的に，製剤からの薬物の溶出性とか含量均一性など，医薬品が本来その機能として具備すべき品質に対しては，GMPにもとづいた厳重な管理システムが構築されており，これらの機能性に問題のある製品が出荷されることは考えにくい状況にある．しかしながら，医薬品の汚染とりわけ異物混入に関しては，数百万錠に1錠の発生であっても問題化することがあり，それはもはや統計的品質管理のおよぶ領域ではなくなっている．このため異物に関してはより厳格な対応が必要となる．今回は医療過誤とあわせて，この部分にも焦点をあててみたい．

2．錠剤の識別性の向上

(1)錠剤の大きさについて

日本国内では径が7～8mmで白色の円形錠が好まれる傾向にあり，欧米の大型で毒々しい色に着色された変形錠が多く市販されている状況とはかなり異なった状況にある．このことは，多分に日本の国民性からくるものと思われるが，錠剤の識別性を低下させる原因となっていることは明らかである．

また，直径が 5mm から 6mm 程度の非常に小型の錠剤も市販されているが，これは昭和 50 年ころに主として開発された製剤であり，その主たる目的は患者の服薬性を向上することにあった．しかしながら高齢化社会を迎えた現在においては，服薬性はもちろんであるが，錠剤のハンドリングのしやすさや識別性・調剤鑑査など，多角的な観点から錠剤の形状を議論することが重要となっている．表1は錠剤のつまみやすさの順位と，錠剤の直径との関係を調査した杉原らの研究結果[1)]であるが，素錠では7～8mm辺り，糖衣錠では8～9mm辺りにつまみやすさの評価の分かれ目があることが示されている．一方，図1に示したように服用のしやすさに関しては，素錠・糖衣錠ともに8mm前後で評価が高く，必ずしも小型錠が飲みやすいとは限らない結果となっている．しかも，小型の錠剤では刻印や印刷も相対的に小さくなるため，識別性の点からも問題の生ずる場合がある．このような背景から，最近では直径が 7～8mm の通常サイズの円形状とするのが一般的となっているようである．

このように，白色で円形の錠剤が多数を占めるわが国においては，錠剤間の識別性が重要な問題となる．すなわち，錠剤が包装から取り出された状態になったあとでも，製品名，主薬含有量，メーカーなどが容易に判別できることは，調剤ミス防止や調剤検査・服薬ミス防止の観点から重要な要件であるといえる．

この識別性を錠剤に付与する方法として，素錠の場合には刻印を施すことが一般的である．刻印される情報としては製品名を表わすコード番号やメーカーの社章，主薬含有量を表わす数字などがある．これらの刻印は錠剤の識別を目的とするものであるから，明瞭に判読できることが必須である．この刻印の明瞭さを確保するためには，刻印の設計，錠剤の処方，製造条件などを細かく検討することが必要となるが，このことを論じた文献はほとんどない．そこで，本講演では若干の実例をまじえながら，刻印錠の設計と圧縮成形についてごく簡単に解説を試みてみたい．

（2）字体の大きな刻印錠

刻印の字体を太く大きくし，刻印の判読性を向上させることは，識別性向上に有用である．図2はその一例であるが，字体を大きくすれば刻印の線はそれに比例して太く深いものとするのが通常である．しかしながら，このような刻印を下杵に配置して打錠を行うと，刻印の欠損が高い頻度で認められることがある．この原因は，錠剤と下杵の刻印が嵌合した状態で錠剤に横方向から力を作用させて打錠機から放出させるとき，その衝撃力が刻印部分に集中するためと説明できる．この場合の対策としては，刻印の深さや角度・Rの再検討により，刻印嵌合部からの錠剤の抜けやすさを改善することが必要となるが，一方，上下の杵の運動軌跡を考慮すると，太く深い刻印を上杵に配置することは，最も簡単で有効な対策であることが推察できる．すなわち，圧縮成形後の上杵は錠剤から垂直方向へ離れてゆくため，錠剤は刻印嵌合部分から無理なく開放され，欠損力が作用することはないと考えられる．実際，上杵に同様の大きな字体の刻印を配した場合，欠損のない鮮明な打刻が可能となった．このように大きくて明瞭な刻印錠を安定して製造するためには，刻印の各設計寸法もさることながら，その刻印を上・下杵のいずれに配するかはきわめて重要な問題となることが多い．

（3）刻印の摩損対策

錠剤が平面錠の場合は問題となることは比較的少ないが，R面である場合は刻印の摩損による識別性の低下に注意が必要である．上下杵で打錠用粉粒体を高速圧縮（動的圧縮）する場合，粉粒体内部での応力分布は液体と違って均一ではなく，上下杵間距離の小さい部分に応力が集中する．したがってR面の錠剤においては，錠剤の周辺部分から中央部に向かって，圧縮力が低下する分布となる．もしもR面の錠剤中央部分に刻印が施されていると，刻印部分は周辺部分よりも弱い圧縮力で成形されることになり，刻印部分の脆性が周辺部分よりも増大する．しかも錠剤の除粉，コーティング，包装などの各操作，および輸送中などに生じる摩擦力や衝撃は錠剤R面中央部に集中する場合が多く，これら2つが相乗して刻印を磨耗させ，不鮮明にする原因となる．したがってR面の錠剤の場合は，2段R面とすることなどにより，刻印面のRをできるだけ大きくして平面に近づけることや，錠剤のR面中央部分を避けて刻印を施すなどの配慮が必要である．

(4)異形錠の設計

市販されている錠剤の多くは円形であるが，一方では円形以外に楕円や四角，花形，蝶型などの異形の錠剤も市販されており，識別性の向上に寄与している．

図3に示したのは，市販されている蝶型錠の形状および寸法であるが，その特異な形状は通常の円形状と比較して非常に注意を引く．この形状について前出の杉原らは，60 人のパネラーに対してイメージ調査を実施している [2]．その結果を引用して表2～表4に示したが，若年者では好意的な結果となっているが，全体としての好意的回答は約半分である．好ましい理由には「印象に残る」や「従来にない形」などが多く，このような形状の識別性が高く，誤認・誤薬の防止，調剤鑑査や服薬指導などに有用であることが明らかとなった．

しかし一方，好ましくない理由としては「蝶が嫌い」というものが認められた [2]．また高齢者になると「服用しにくい」という回答が増加する傾向にあり，対象とする患者の年齢層を意識した形状の検討も必要であることが示唆されている．ところで，この蝶型の形状の主目的は識別性以外に分割性の向上という面もある [46]．分割性の正確さも投薬上非常に重要である．この蝶型は従来の円形の割線錠では正確な分割が困難であること，および分割精度における個人差が大きいことなどに着目し [3～7]，割線部分にくびれをつけてより正確な分割ができるよう検討された形状である．表5は，円形で割線のある直径 7mm の通常の錠剤と，ここで述べている蝶型の錠剤を，年齢ごとに男女それぞれ 1 名ずつのパネラーに分割してもらった結果を示したものであるが，明らかに蝶型の形状の分割精度が優れた結果となっている．

分割のしやすさに関する意識調査でも蝶型錠は 100%分割しやすいという結果となっている．また，分割後の錠剤に対して15局質量偏差試験法を適用して判定値を算出した場合，蝶型錠では余裕を持って適合する結果となっているが円形錠では若干不安の残る結果となっている．

(5)刻印とスティッキング対策

刻印を不明瞭とする要因の中で重要なものがスティッキングである．スティッキングとは，錠剤と杵表面との接触面における付着力が大きくなって，錠剤の表面部分が一部はがれて杵面に付着する現象をいう．スティッキングのレベルは，錠剤の光沢がわずかに失われる程度の軽微なものから，錠剤全面が剥離するような重度のものまでさまざまである．このスティッキングが発生するのは，ステアリン酸マグネシウムなどの滑沢剤不足や主薬成分の物理化学的性質，あるいは造粒条件をはじめとする製造条件の不適切に起因する場合が大部分であって，それらの改善が根本的な解決手段となる．しかしながら，スティッキングの発生状態を注意深く観察すると，特定の刻印の特定の位置で発生し始めることが多い．これは，刻印自体にもスティッキングを誘発する要因が存在することを示しているが，具体的には，図4に斜線で示すようなアルファベットのAやB，そして数字の4や8などの中州状態の部分でスティッキングが発生しやすい．この部分は周囲から孤立した状態となっているため，他の部分よりも剥離しやすいことは容易に想像できる．したがって刻印を設計する場合には，数字やアルファットの字体に注意し，できるだけ中州状の部分の面積を広くする工夫などが必要である．

3. 製造工程における医薬品の汚染防止対策

日本においては異物混入についての市場の関心が高く，また製薬企業自身も医薬品は清浄であるべきとの立場に立って，異物混入対策に真剣に取り組んでいるところが多い．

一般に，医薬品製剤への異物混入防止対策は次の3つの原則に要約される．

- 異物を製造工程に持ち込まない
- 製造過程で異物を混入させない
- それでも製品中に混入してしまった異物は除去する

これらについて順番に解説する．

(1)原料，中間品中の異物混入防止対策

一般に固形製剤の場合，液剤とは違って，一旦混入した異物を検知，除去することは相当に困難であるし，手間がかかる．従って，原料メーカーにおける異物混入防止対策が重要なポイントとなる．そのために原料メーカーと購入契約を結ぶ際には，混入している異物の質と量を把握し，品質規格とベンダーオーディットなどに関する条件を契約条項に組み入れ，原料メーカーの工程と品質の確認を定期，不定期に実施することが肝要であり，問題が起これば，その都度，異物の分析結果を原料メーカーに報告し，原因の究明と適切な対策の実施を依頼する必要がある．

しかしながら，賦形剤などの副原料に関しては，食品産業など他用途のものを一部医薬用に転用している場合があり，医薬用としての要求品質が必ずしも満足されているとはいえない場合が起こりうる．この意味においては，医薬品用添加物に対するGMP基準の制定は医薬品の品質向上に繋るものとして大いに期待されてよい．ところが一方，医薬品産業が使用する原料は他用途よりもはるかに少量である場合が多く，原料メーカーにとって医薬品産業は必ずしも重要な顧客ではないということも無視できない事実であって，このことは，原料メーカーが対応できる原料品質と医薬品メーカーが要求する品質に大きな差が生じた場合に問題が起こりうることを示唆するものといえる．

一方，外国においては，たとえ主薬原料といえども概して異物混入に鷹揚な場合が多く，混入異物に対して問題点を指摘しても速やかな解決に至る例は比較的少ない．このことが，安価な医薬品原料を国外に求めにくくし，製造原価低減という企業の基本を犠牲にする原因ともなっており，異物品質に関する国際調和が今後大いに進展することが要望される理由となっている．

(2)篩による異物の除去

固形製剤用の原料粉末や中間製品である製錠用顆粒あるいは製品としての顆粒剤，細粒剤などに混入する異物を除去する方法として篩過処理が通常よく行なわれるが，篩過の原理を考えれば異物除去の効果が原料粉末や中間製品より大きい異物に限定されることは明らかである．また，異物が微細な粒子の集合体である場合，篩を通す過程で微細な粒子にほぐれて，異物汚染を拡散させてしまうことがあるので注意を要する．

異物を除去することを目的とする以上，篩の目開きの選択はできるだけ細かくする必要があるが，その限度は適用する原料粉末や中間製品の粒子サイズと凝集性など篩の通過し易さに関係する特

性と篩過装置の機構に依存する.

図5に2種類の篩過装置を示したが, (a)はスクリーンが振動するタイプの篩過装置であり, 付着凝集性の強い粉末でもスクリーンが目詰まりしにくく, かつ篩過能力が高いという特徴がある. 一方, 筆者らの固形製剤の製造工場では, ブラシで圧篩する方式の篩過装置(b)が多用されており, (a)のタイプと同様に比較的凝集性の強い粉末でも実用的な処理速度が得られ, かつ篩の目開きを細かく選定できる. しかしながら(a), (b)いずれのタイプにせよ, 異物が微細な粒子の集合体である場合は, 上述のとおりかえって異物汚染を拡散させる危険性があることに注意が必要である.

また一方, 篩で異物を除去する場合には, 細長い異物が篩を「縦通過」することを考慮しておく必要がある. たとえば, その典型的な例である毛髪の場合, その径はおよそ100μm であるから, 通常使用される目開き300～500μm の篩を縦に通過してしまう確率は高い. したがって篩による異物除去においては対象とする異物の形状を充分検討しておくことが必要である.

異物除去を目的とする篩過工程では, ロット毎あるいは1日処理毎に, 篩上に捕捉された異物を透明粘着テープに転写・固定して品名・ロット番号・処理量・処理条件などとともに工程管理記録にファイルしておくことが重要で, このことにより異物の増減のトレンドや新規な異物の混入の有無などが確認できる. また, 必要に応じて, 顕微鏡写真やFTIR, EPMA分析を行なえば原料メーカーへのフィードバックや異物の混入経路の推定に大きく役立つ.

以上のように, 篩による異物の除去はある限定された条件下で効果を発揮するものであるから, 篩過装置の形式と条件設定の選定にあたっては篩過対象と除去すべき異物の特性を充分見極めておくことが必要で, 要時, チャレンジテストでその異物除去能力を評価することも肝要と言える.

(3)マグネットによる磁性異物の除去

固形製剤用原料粉末や中間製品中の磁性を有する異物除去の方法として, 製造工程中の粉粒体の通過経路にマグネットを設置し, その磁力によって磁性異物を捕捉する方法が採られる. 具体的には, 図6に示すように, 複数のマグネット棒を粉粒体の通過経路中に設置して, その間に粉粒体を流し, 混入する磁性異物たとえば鉄粉やステンレス摩耗片などをマグネット棒に捕捉する方法である. この方法によれば, 篩過では対応できない非常に細かい磁性異物の除去が可能となる. しかし, この方法には折角捕捉された磁性異物が粉粒体の流れによって削り取られ, 粉粒体中に再混入してしまう問題があり, このことを避けるためには粉粒体の流れの速度を落とすなどの対策が必要となる.

異物除去用に使用されるマグネットの強度は通常 6000～12000 ガウス程度であるが, 強ければ強いほど磁性異物捕捉効果が上昇し, かつ捕捉異物の粉粒体による削り取られも減少するが, マグネットを強くするとその確実な固定方法が必要となるし, マグネット同志の, あるいは磁性を有する機械部品との間の強力な接着力のために, 分解, 組み付けなどのハンドリングにおいて問題が生じる可能性もあるので注意が必要である.

マグネットによる磁性異物除去の日常の管理としては, 篩過の場合と同様にロット毎あるいは1日処理毎に分解して, 捕捉された異物を透明粘着テープに転写・固定して管理記録を作成しておくことが重要で, このことにより磁性異物の混入量の推移を把握でき, 磁性異物が異常に増加した場合

などに，原料メーカーへのフィードバックがタイムリーに実施できるし，自工程の異常の検出もより正確に行える．

この磁石による異物除去方法は相当に微少な異物に対応できる反面，磁性の無い異物に対しては対応できない．一方，前述の篩過方法の場合は非磁性異物には対応できるもののスクリーン目開き以下の異物には対応できない．つまり，それぞれに一長一短がある訳であり，これらを組み合わせて使用すればある程度それぞれの短所を補完し合うことが期待される．換言すれば，異物対策においては適用可能な除去手段を組み合わせて使用することが必要であるといえる．

(4)風篩式異物除去装置による異物の除去

前節において既に述べたことであるが，固形製剤をはじめとする医薬品の製造においては，異物の混在しない原料を確保し医薬品の汚染に対して充分にバリデートされた製造プロセスを確立することが異物対策の原則となる．しかし，輸入原料などでは異物混在の危険性が高い場合もあり，その対策として一般には原料の前処理(篩過)や最終製剤の外観検査が実施されている．しかし混入した可能性のある異物は可能な限り早期に除去する必要があることから，粉粒状の中間製品に混在する異物を除去する目的でシオノギが開発した連続式インライン型異物除去装置[8)]を簡単に紹介してみたい．

(4.1) 異物除去装置の概要

装置の概略を図7に示した．図の(A)は流動層型異物除去装置で，粒度の揃った顆粒剤に混在する異物の除去を目的とし，一方(B)のトンネル型異物除去装置は微粉を含む打錠用あるいはカプセル充填用粉粒体を対象とする装置である．これらの装置は，底面からの流動化空気により供給される粉粒体を流動状態にさせ，粉粒体と混在異物との物性差や磁性などを利用して混在異物を分離除去するものである．具体的には金属，ガラス片等の重質異物は落下捕集，ビニル片等軽質異物は排気除去し，磁性異物は磁石により捕集する．なお，流動化空気速度，粉粒体供給速度，排出口高さは対象とする粉粒体の物性により適宜設定を行い，必要に応じて装置内に仕切板の設置，磁石本数の変更も可能である．

(4.2) 本装置の異物除去原理

(4.2.1) 顆粒と重質異物の流動化開始速度差

流動化開始速度とは，充填された粒子層の下から空気を送風する時，徐々に風速を増加させて粒子が運動しはじめる瞬間の送風速度のことであり，簡易的に(1)式[9,10)]により求めることができる．

$$umf = 0.7\{\rho(\rho_p - \rho)\}^{0.94} Dp^{1.82} / \rho u^{0.88} \qquad (1)$$

ここで ρ および ρ_p は流体と粒子の密度，μ は流体粘度，Dp は粒子径である．異物として金属を想

定し，顆粒との流動化開始速度の違いを(1)式より計算して図8に示した．なお，顆粒および金属(SUS)の密度はそれぞれ 1.0 , 7.8 g/cm^3 とした．図8では両者間の流動化開始速度に大きな差が認められ，この差を利用すれば金属等の重質異物を粉粒状の製剤中間製品から分離除去できると期待される．

(4.2.2) シミュレーションによる異物分離機構の解析

離散要素法を用いた粒子運動の数値シミュレーションは，ドラム混合[11]や錠剤コーティング[12]など固形製剤分野においても現象の解析に広く用いられるようになっている．この手法を用いて流動層内における粒子の運動をシミュレートし，今回開発した異物除去装置の異物分離機構を解析した．

個々の粒子の運動は離散要素法を用いた Lagrange 的な手法により追跡し，また流体に対しては速度，圧力などを空間的に重み付けをして平均化した局所平均量を用いた基礎式を解くことにより，粒子の流動化現象をシミュレートした．具体的には，バネ，ダッシュポットおよび摩擦スライダを用いた Cundall,Strack[13]と同様のモデルにより粒子間の接触力を与え，この接触力と流体から受ける力をもとに個々の粒子の運動を Lagrange 的に計算し，流体の運動については粒子の存在を考慮した連続の式および運動方程式を計算する2way coupling 法[14]を用いた．なお，計算は球粒子を用いた2次元流動層を対象とし，直径が等しく密度の異なる 2 種類の粒子の混合物(軽質:重質=96:4)を，流動層の底面から一様な流動化空気で吹き上げた時の粒子の運動をシミュレートした．計算条件を表1に示したが，特に送風速度は顆粒の流動化開始速度(1.5 m/s)の1.2倍(1.8 m/s)とした．

図9は送風開始からの粒子のスナップショットを経時的に示したものである．黒丸が重質粒子を表し，初期状態では斜めにほぼ整列させて配置した．図9(1)～(6)に示すように流動化が始まるとともに，重質粒子は流動化する軽質粒子の空隙を落下するように移動することがわかる．これは上述の流動化開始速度による推定と一致する現象であり，さらに時間が経過すると重質粒子は図9(7)～(9)に示すように底面近くで周囲の顆粒とともにほとんど静止した状態となった．この時の流動化は粒子層の上層部のみで起こっており，沈降した重質粒子は再び浮遊することはなく，軽質粒子と重質粒子の分離状態が保たれた．これは重質粒子が含まれる部分(下層部)と軽質粒子のみの部分(上層部)の見かけのかさ密度に差が生じ，この差によって流動状態に差違が生じたためであるといえる．以上のように2次元流動層を用いて本装置の異物分離機構をシミュレートした結果，粒子径が等しい場合，軽質粒子と重質粒子とを完全に分離可能であり，本装置の有用性が示唆された．

(4.3) 本装置の異物除去能力の実験的評価

上述のシミュレーションで示された本装置の異物除去能力を実際に確認した結果，図7に示すように顆粒と金属異物との物性差による除去限界は SUS 粒子径 0.1～0.2mm 程度であり，顆粒の供給速度が小さいほど除去能力は向上した．このことからシミュレーション結果で可能性が示唆された密度差を利用する顆粒と金属異物の分離方法が実際に可能であることが実験的に証明されたといえる．そして異物が SUS の場合その分離限界は顆粒の大きさの 1/3～1/2 程度であることがわかった．なお，ここでは省略するがトンネル型についても同様な結果が得られている．

以上の検討結果をもとに 6000 ガウス磁石を標準仕様とした装置を生産ラインに組み込んだ結果，安定した異物除去機能が得られ，その有用性が証明された．図10は生産プロセスでの使用例を示したものであるが，最近はプロセスのクローズド化，省人化を目的とした空気輸送ラインが多く，インラインで使用できる本装置の有用性は大きいといえる．ちなみに，図11は生産ラインで実際に捕集された異物であるが，内部に微小金属を伴った顆粒などもあり，従来の方法(篩過や外観検査機)では除去困難な異物の除去も可能となったことが確認された．以上から，本装置はシオノギの固形製剤工程に標準装備されることとなった[15,16]．

5．外観検査機の導入

以上のように原料や製造工程，中間製品などに細心も注意はらっても，外来異物の混入リスクを完全になくすことは困難であり，錠剤や顆粒剤，カプセル剤など最終の工程が完了した時点で外観検査を実施することが常識となっている．最近では，各メーカーから高性能な外観検査機が市販されているが，これらの装置は錠剤やカプセル剤などの搬送方法は異なるものの，検査の方式そのものはほぼ同じであると言ってよい．検査原理を図12に示す．すなわち，錠剤をカメラで撮像した場合，錠剤表面に異物が存在すると，その部分の明るさや，明るさの差分に変化が生じる．この変化があらかじめ設定されている閾値を越えると，異物であるという判定となり，その錠剤は系外へ排除される．高性能の検査機では一時間あたり 10 万錠以上の検査が可能であり，異物除去や外観品質の確保に大きな威力を発揮している．

しかしながら，最終工程にこのような検査機があるからといって，上述してきた中間工程や原料に対しての異物混入防止の取り組みがおろそかになっては本末転倒であり，最終工程におけるこれらの外観検査機は，製造工程全体が異物混入に対して良好なコントロール状態にあることを検証するものであるという考え方が必要である．

6．おわりに

医薬品の医療過誤防止や健康被害防止について，医薬品メーカー側からの取り組みの一端を紹介させていただいたが，これらの取り組みは合理的な製剤設計およびGMPに則った医薬品製造プロセスと協調していることが重要である．

今回の講演により，別の演者が紹介した包装仕様の問題も含めて，製造，物流，調剤，投薬の各ステップのいずれの段階においても医療過誤や健康被害が起きないよう，我々医薬品メーカーがたゆまぬ努力を続けていることをご理解いただければ幸いである．

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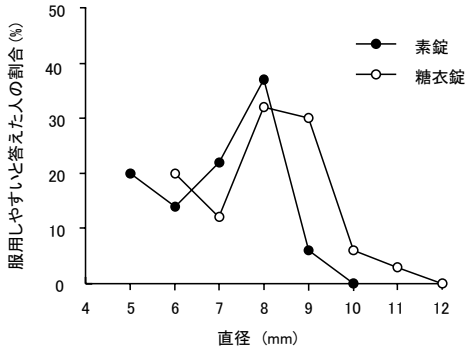


杉原ら「剤形および包装における識別性の検討」 病院薬学 Vol. 12, No.4 (1986)

図 1. 錠剤の服用しやすいサイズ

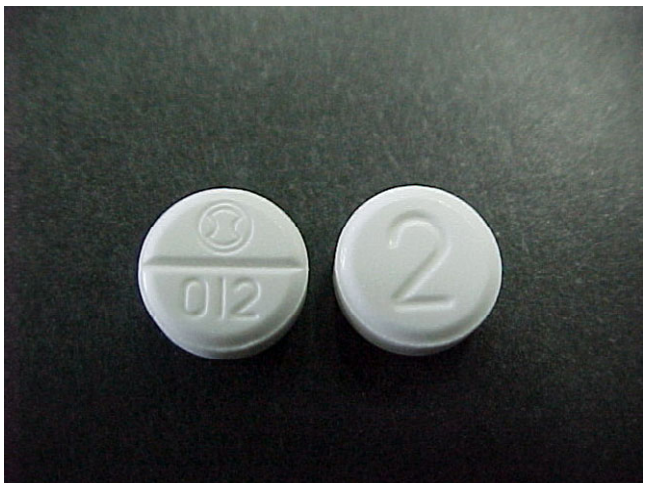


図2 字体の大きな刻印錠

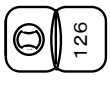
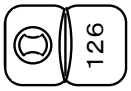
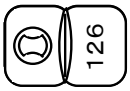
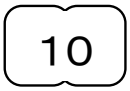
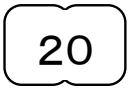
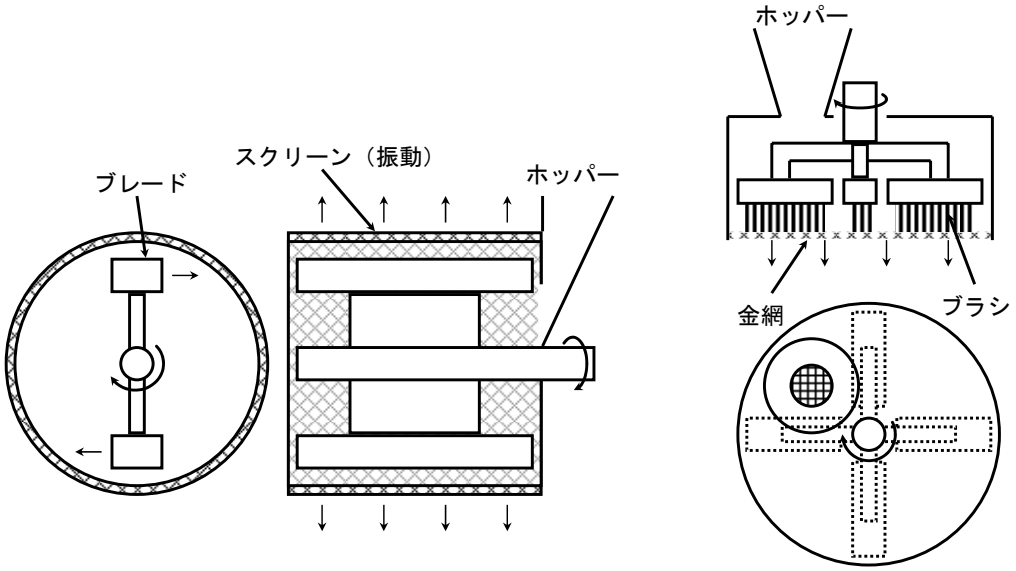
		5mg 錠	10mg 錠	20mg 錠
形状	表面			
	裏面			
	側面			
寸法	長径	約 7.5 mm	約 9.3 mm	約 9.3 mm
	短径	約 4.5 mm	約 5.6 mm	約 5.6 mm
	厚さ	約 2.4 mm	約 3.3 mm	約 3.5 mm

図 3. 蝶型錠の形状と寸法

図 44. スティッキングを誘発しやすい刻印部分



(a) 振動式篩過装置　　(b) 回転ブラシ式篩過装置

図5. 2種類の篩過装置

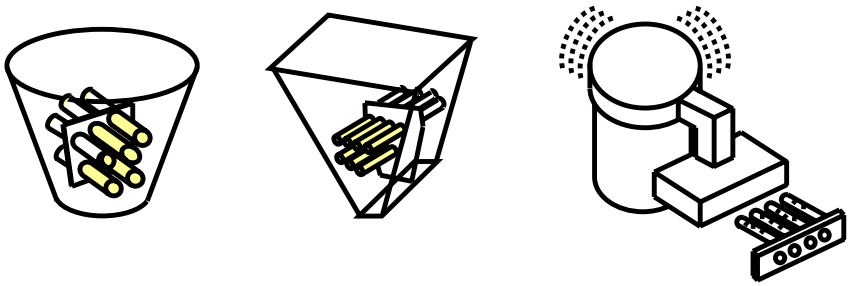


図6　　粒体の通過**経路**に設置したマグトット**棒**

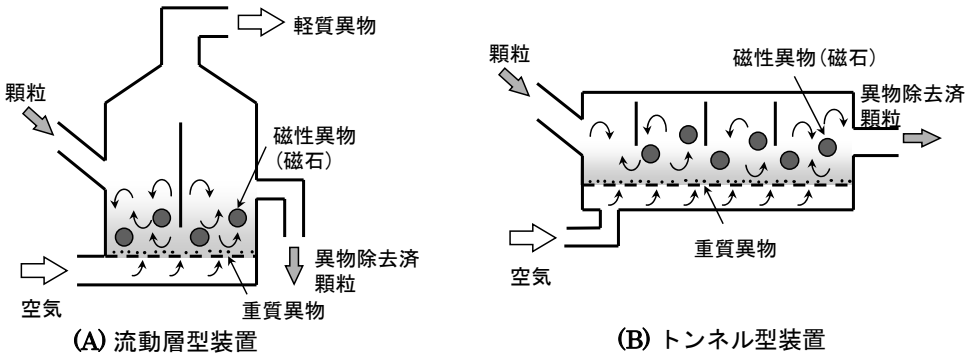


図７　風篩式異物除去装置の概略

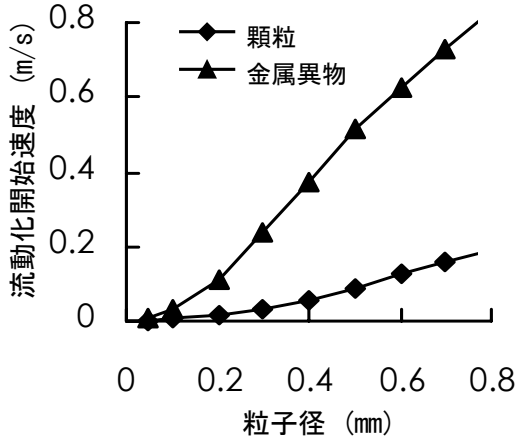


図８　流動化開始速度と粒子径の関係

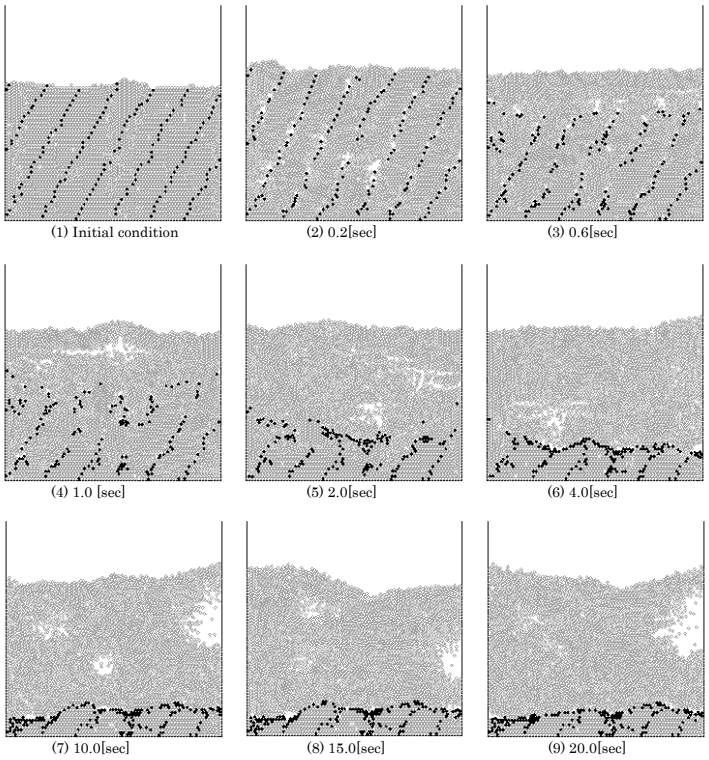
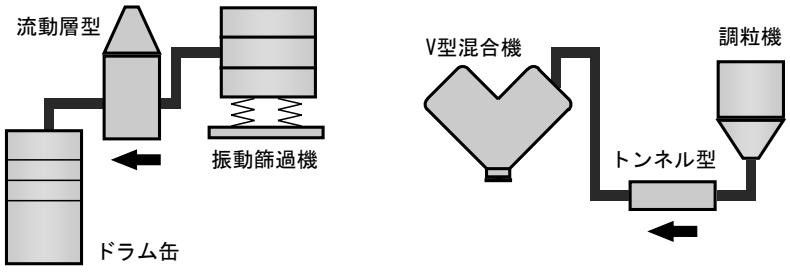


図９ シミュレーション結果



・篩過後の収缶ラインに設置　　・調粒後の空気輸送ラインに設置

図10 生産プロセスでの使用例

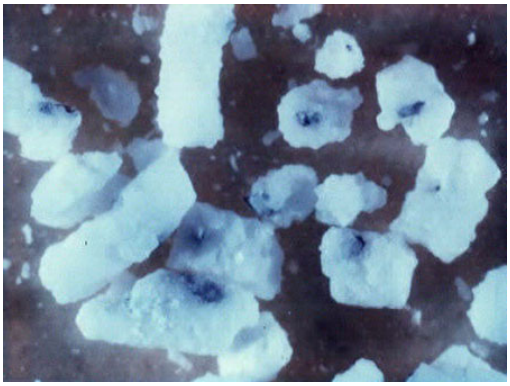


図11 捕集異物の例（異物を伴った顆粒）

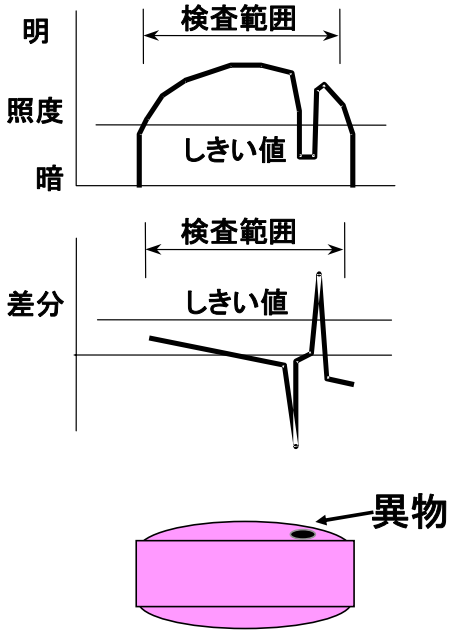


図12. 外観検査機の検査原理

表 1. 錠剤のつまみやすさの順位

素錠		糖衣錠		
直径	平均順位	直径	平均順位	
11 mm	2.4	10 mm	2.4	
10 mm	2.4	11 mm	2.5	
9 mm	2.6	12 mm	2.7	⇧ つまみやすい
8 mm	3.2	9 mm	3.1	
7 mm	4.6	8 mm	4.5	⇩ つまみにくい
6 mm	5.9	7 mm	5.8	
5 mm	6.8	6 mm	6.9	

杉原ら「剤形および包装における識別性の検討」病院薬学 Vol. 12, No.4 (1986) 一部改

表 2 蝶形錠剤のイメージは好ましいか否か

年齢	回答者	好ましい	好ましくない	なんとも言えない
20代	学　生 (10)	6	2	2
	会社員 (10)	4	5	1
	薬剤師 (20)	7	6	7
	合計 (40名)	17	13	10
		42.5%	32.5%	25.0%
30代	薬剤師	2	2	4
	(8名)	25.0%	25.0%	50.0%
40代	薬剤師	0	1	0
	(1名)	0.0%	100.0%	0.0%
50代	薬剤師	0	1	0
	(1名)	0.0%	100.0%	0.0%
60代	薬剤師	2	6	2
	(10名)	20.0%	60.0%	20.0%
合計	60名	21	23	16
		35.0%	38.3%	26.7%

日高ら「変形錠剤の使用性について」病院薬学 Vol. 18, No.4 (1992)

表 3. 蝶形錠剤のイメージの好ましい理由

年齢	回答者	美しい	印象に残る	明るい	従来にない形	その他	その他の内容
20代	学　生 (10)	0	5	0	1	1	形がシンプル
	会社員 (10)	0	2	1	1	0	
	薬剤師 (20)	0	5	2	2	2	分割しやすい 飲むのが楽しい
	合計 (40名)	0	12	3	4	3	
		0.0%	30.0%	7.5%	10.0%	7.5%	
30代	薬剤師	0	1	0	1	1	割線があるので使いやすい
	(8名)	0.0%	12.5%	0.0%	12.5%	12.5%	
40代	薬剤師	0	0	0	0	0	
	(1名)	0.0%	0.0%	0.0%	0.0%	0.0%	
50代	薬剤師	0	0	0	0	0	
	(1名)	0.0%	0.0%	0.0%	0.0%	0.0%	
60代	会社員	0	0	0	1	1	分割服用できる
	(10名)	0.0%	0.0%	0.0%	10.0%	10.0%	
合計	60名	0	13	3	6	5	
		0.0%	21.7%	5.0%	10.0%	8.3%	

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表 4. 蝶形錠剤のイメージの好ましくない理由

年齢	回答者	理由（内容）	
20代	学　生	蝶が嫌い	1
	（10名）		
	会社員	形が変	3
	（10名）	服用しにくい	1
		蝶と関係	1
	薬剤師	蝶が嫌い	2
	（20名）	形が変	3
30代	薬剤師	服用しにくい	1
	（8名）	蝶が嫌い	1
40代	薬剤師	蝶が嫌い	1
	（1名）		
50代	薬剤師	蝶が好きではない	1
	（1名）		
60代	会社員	蝶が嫌い	1
	（10名）	服用しにくい	3
		無関係	2
合計	60名	蝶が嫌い	7
		服用しにくい	5
		形が変	6
		蝶と関係ないから	2
		その他	1

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表 5 錠剤の分割性の比較

		蝶型錠（106mg）		7mmΦ円形割線錠（120mg）	
年齢		男性	女性	男性	女性
20代	$\bar{X}$ (mg)(n=40)	52.82	53.03	59.35	60.07
	max (mg)	56.63	55.18	64.53	67.19
	min (mg)	49.60	50.77	52.92	53.02
	c. v. (%)	3.90	2.22	4.56	6.55
	判定値 (%)*)	4.16, 6.11, 5.25, 6.19	2.62, 2.73, 3.72, 3.22	8.89, 5.78, 6.30, 8.52	8.73, 10.03, 12.60, 9.75
	分割しやすさ	○	○	×	×
30代	$\bar{X}$ (mg)(n=40)	53.13	52.96	59.81	59.62
	max (mg)	56.05	57.34	68.97	67.79
	min (mg)	49.18	48.77	51.10	51.49
	c. v. (%)	3.05	3.55	8.12	7.55
	判定値 (%)*)	4.39, 4.02, 4.55, 4.41	7.25, 3.72, 6.31, 2.77	14.96, 11.63, 11.42, 11.72	8.52, 12.63, 12.10, 14.19
	分割しやすさ	○	○	×	×
40代	$\bar{X}$ (mg)(n=40)	53.03	52.84	59.16	59.24
	max (mg)	58.64	55.04	66.67	64.69
	min (mg)	48.32	50.13	50.53	55.16
	c. v. (%)	3.99	2.57	7.34	3.42
	判定値 (%)*)	3.38, 3.71, 6.95, 6.88	3.91, 2.76, 3.77, 3.21	10.88, 8.75, 12.78, 12.32	4.81, 3.71, 7.38, 6.07
	分割しやすさ	○	○	×	×
50代	$\bar{X}$ (mg)(n=40)	52.91	53.01	58.95	59.30
	max (mg)	57.83	56.16	69.80	67.99
	min (mg)	47.64	49.28	50.84	53.13
	c. v. (%)	4.55	3.26	7.90	6.19
	判定値 (%)*)	6.28, 7.25, 5.16, 6.49	4.44, 5.17, 3.05, 4.59	15.91, 12.36, 12.90, 10.12	11.28, 10.60, 10.36, 8.13
	分割しやすさ	○	○	×	×

*）40個のサンプルを無作為に10個ずつ抽出しそれぞれについてJP15重量偏差試験の判定値を算出した.

[Proceeding -4]

A Comparison of Neuroprotective Efficacy of Newly Developed Oximes (K203, K206) and Commonly Used Oximes (Obidoxime, HI-6) in Tabun-Poisoned Rats

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Abstract

The neuroprotective effects of newly developed oximes (K203, K206) and commonly used oximes (obidoxime, HI-6) in combination with atropine in rats poisoned with tabun at a sublethal dose (180 μg/kg i.m.; 80% LD_{50}) were studied. The tabun-induced neurotoxicity was monitored using a Functional observational battery and an automatic measurement of motor activity. The neurotoxicity of tabun was monitored at 24 hours and 7 days following tabun challenge. The results indicate that only K203 and obidoxime in combination with atropine allow all tabun-poisoned rats to survive within 7 days following tabun challenge while two non-treated tabun-poisoned rats and one tabun-poisoned rat treated with K206 or HI-6 in combination with atropine died within 7 days. Only one of newly developed oximes (K203) combined with atropine seems to be effective for a decrease in tabun-induced neurotoxicity within 24 hours after tabun sublethal poisoning although it is not able to eliminate tabun-induced neurotoxicity completely. On the other hand, the neuroprotective efficacy of commonly used oximes (obidoxime and HI-6) as well as one of newly synthesized oxime (K206) is significantly lower in comparison with K203 according to the number of eliminated tabun-induced neurotoxic signs at 24 hours after tabun challenge. Due to its neuroprotective effects, K203 appears to be suitable oxime for the antidotal treatment of acute tabun poisonings.

Introduction

Tabun (GA; O-ethyl-N,N-dimethyl phosphoramidocyanidate) belongs to highly toxic group of organophosphorous compounds misused as chemical warfare agents for military as well as terroristic purposes. Toxic effects of tabun are extraordinarily difficult to antagonize because of conformational changes of acetylcholinesterase-tabun complex in the acetylcholinesterase (AChE, EC 3.1.1.7) gorge

Key words: tabun, atropine, K oximes, HI-6, obidoxime, functional observational battery,

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that make the nucleophilic attack of oximes almost impossible [1]. Tabun appears to produce centrally mediated seizure activity that rapidly progresses to status epilepticus and contributes to profound brain damage. Thus, the exposure of experimental animals to tabun in convulsions-induced doses may result in irreversible lesions in the central nervous system (CNS) that can be manifested as behavioral effects in convulsant survivors [2]. Therefore, the ability of antidotes to block the acute neurotoxic effects of tabun and prevent the development of irreversible lesions in CNS is important for successful antidotal treatment. The oximes exert more potent effects in the peripheral compartment compared to central system due to their poor penetration into CNS. Nevertheless, there are published results demonstrating the penetration of oximes into CNS and subsequent reactivation of nerve agent-inhibited AChE in the brain [3,4]. Although the rate of reactivation of nerve agent-inhibited AChE in the brain is lower compared to peripheral compartment, the role of reactivation of nerve agent-inhibited AChE in CNS is important for survival from nerve agent exposure [5]. As the ability of currently used monopyridinium (e.g. pralidoxime) and bispyridinium oximes (e.g. obidoxime, HI-6) to counteract adverse effects of tabun is generally low [6], the replacement of commonly used oximes (pralidoxime, obidoxime) as well as H oximes (the oxime HI-6) with a more effective oxime has been a long-standing goal for the treatment of tabun poisoning. For this reason, new bispyridinium oximes: K203 (E)-1-(4-carbamoylpyridinium)-4-(4-hydroxyimino- methylpyridinium)-but-2-ene dibromide and K206 (*E*)-1-(3-carbamoylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide (Figure 1) were developed to improve the efficacy of antidotal treatment in reactivating tabun-inhibited AChE and eliminating tabun-induced acute lethal toxic effects [7]. The aim of this study was to evaluate the potential neuroprotective effects of currently available oximes (obidoxime, HI-6) and newly developed oximes (K203, K206) in combination with an anticholinergic drug atropine in tabun-poisoned rats. The tabun-induced neurotoxic signs were determined using a Functional observational battery (FOB), a non-invasive and relatively sensitive type of neurological examination for a wide range of neurobiological functions. FOB consists of 47 measurements of sensory, motor and autonomic nervous functions. Some of them are scored, the others are measured in absolute units [8,9].

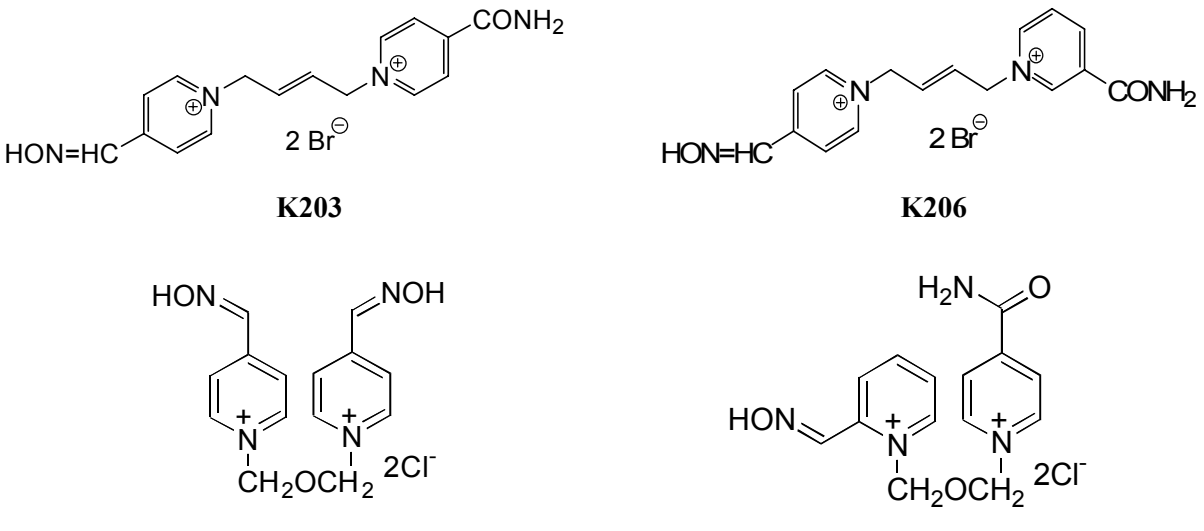

Figure 1 Chemical structure of oximes studied

Material and Methods

Male albino Wistar rats weighing 180-200g were purchased from Konarovice (Czech Republic). They were kept in an air-conditioned room (22 ± 2 °C and 50 ± 10% relative humidity, with lights from 7.00 to 19.00 hours) and allowed access to standard food and tap water *ad libitum*. The rats were divided into groups of 8 animals. Handling of experimental animals was done under the supervision of the Ethics Committee of the Faculty of Military Health Sciences in Hradec Kralove (Czech Republic).

Tabun was obtained from Military Technical Institute in Brno (Czech Republic) and was 95% pure as assayed by acidimetric titration. Obidoxime, the oxime HI-6 and newly developed oximes (K203, K206) of 98.0% purity were synthesized earlier at the Department of Toxicology of the Faculty of Military Health Sciences in Hradec Kralove (Czech Republic). Their purity was analyzed using HPLC. All other drugs and chemicals of analytical grade were obtained commercially and used without further purification. All substances were administered intramuscularly (i.m.) at a volume of 1 mL/kg body weight (b.w.).

Tabun was administered at a sublethal dose (180 μg/kg b.w. - 80% LD_{50}). One minute following tabun challenge, the rats were treated with atropine (21 mg/kg b.w.) in combination with an oxime. The oximes were administered at equitoxic doses corresponding to 5% of their LD_{50} values: HI-6 (39 mg/kg b.w), obidoxime (10.5 mg/kg b.w), K203 (16.3 mg/kg b.w) and K206 (19.3 mg/kg b.w). The neurotoxicity of tabun was monitored using the FOB at 24 hours and 7 days following tabun poisoning. The evaluated markers of tabun-induced neurotoxicity in experimental animals were compared with the parameters obtained from control rats that saline was administered instead of tabun and antidotes at the same volume (1 mL/kg b.w.). Data collected with the FOB include categorial, ordinal and continuous values. Their statistical analyses were performed on a PC with a special interactive programme NTX [8]. The categorial and ordinal values were formulated as contingency tables and judged consecutively by Chi-squared test of homogeneity, Concordance-Discordance test and Kruskal-Wallis test, respectively. The continual data were assessed by successive statistical tests: CI for Delta, Barlett test for Equality of Variance, Williams test and Test for Distribution Functions. The results of experimental groups were compared to the results from control rats. The differences were considered significant when $p < 0.05$.

Results

The results of the experiments related to the measurement of tabun-induced neurotoxicity at 24 hours and 7 days following tabun poisoning are summarized in Table 1 and 2. Two non-treated tabun-poisoned rats and one tabun-poisoned rat treated with K206 or HI-6 in combination with atropine died within 7 days following tabun administration. Only K203 and obidoxime in combination with atropine allow all tabun-poisoned rats to survive until the end of experiment (7 days folowing the intoxication).

The evaluation of tabun-induced neurotoxic signs at 24 hours following intoxication proved significant alteration of 22 observed parameters. Tabun produced passive behavior of rats during handling and retention and an increase in the value for miosis and nose secretion. Gait and mobility were somewhat impaired and the level of unprovoked activity was reduced. In addition, no reaction

during a reflex testing consisting of recording each rat`s response to the frontal approach of the blunt end of a pen or a touch of the pen to the posterior flank was found. No ability of pupils to constrict in response to light was demonstrated either. A significant decrease in forelimb and hindlimb grip strength, food receiving, body temperature and spontaneous horizontal as well as vertical motor activity were also observed at 24 hours following tabun challenge. The newly developed oxime K203 in combination with atropine was able to prevent many tabun-induced signs of neurotoxicity observed at 24 hours following tabun challenge with the exception of an increase in the value for miosis, no ability of pupils to constrict in response to light, a decrease in forelimb grip strength, body temperature, food receiving and spontaneous motor activity. Another newly developed oxime K206 as well as both commonly used oximes (obidoxime, HI-6) seem to be significanly less efficacious than K203 because they were not able to eliminate passive behavior of rats during handling and catching, the impairment of gait and mobility, a decrease in rat`s response to the frontal approach of the blunt end of a pen and to auditory click stimulus aa well as a decrease in the grip strength of all limbs (Table 1).

The 7 days neurotoxicity evaluation proved successive subsiding of neurotoxicity signs. The evaluation of tabun-induced neurotoxic signs at 7 days following intoxication showed a significant alteration of 4 observed parameters only. Therefore, the differences in neuroprotective efficacy of all oximes studied at 7 days after tabun poisoning are not apparent (Table 2).

Discussion

Currently available obidoxime is able to partly eliminate tabun-induced acute neurotoxicity following i.m. administration of tabun at a sublethal dose, nevertheless, its neuroprotective efficacy is not satisfactory [10]. The oxime HI-6, that was developed and introduced by some countries for the antidotal treatment of severe acute soman poisonings because of its higher reactivation and therapeutical efficacy compared to currently used oximes such as pralidoxime and obidoxime [11], was demonstrated to be significantly less efficacious to block tabun–induced acute neurotoxicity than obidoxime [10]. The unsatisfactory efficacy of above mentioned oximes to eliminate tabun-induced acute neurotoxicity is possible to explain due to low potency of oximes in reactivating tabun-inhibited AChE *in vitro* and *in vivo* [12,13]. Therefore, new oximes have been developed to increase the reactivating potency as well as neuroprotective efficacy of antidotal treatment of acute tabun poisonings. Some new bispyridinium AChE reactivators able to reactivate AChE inhibited by tabun and counteract tabun-induced acute neurotoxicity have been still developed but without extraordinary effects [14]. Based on the structure-activity relationship study, there are five most important structural factors influencing the affinity of AChE reactivators toward nerve agent-inhibited AChE and subsequent oxime reactivity [15]: presence of the quaternary nitrogen in the reactivator molecule, length of the connection chain between two pyridinium rings, presence of the oxime group, position of the oxime group at the pyridinium ring and number of oxime groups in the reactivator structure. Above mentioned data on a reactivator structure allowed us to postulate requirements on the structural parameters of new reactivators of tabun-inhibited AChE.

Our results demonstrate that only one of newly developed oximes studied (K203) appears to be significantly more effective to eliminate tabun-induced acute neurotoxicity in rats in comparison with currently available oximes (obidoxime, HI-6). On the other hand, the potency of another newly

Table 1 The values of tabun-induced neurotoxic markers measured at 24 hours following tabun challenge by the Functional observational battery (No 1-11, 15-36, 44 - scored values, No 12-14, 37-43, 45-47 - values in absolute units). Statistical significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ - comparison with the control values (applied abbreviations: RRF, air righting reflex; RRV, air righting reflex from vertical position; x/M, average or modus value; ± s, standard deviation; A, atropine; n, number of surviving animals).

24 hours		Controls		Tabun+A+K 203		Tabun+A+K206		Tabun+A+HI-6		Tabun+A+obidoxime		Tabun	
No.	Marker	x/M	±s	x/M	±s	x/M	±s	x/M	±s	x/M	±s	x/M	±s
1	Posture	1,00		1,00		**3,00*****		**3,00*****		**3,00*****		**3,00*****	
2	Catch difficulty	2,00		2,00		**1,00*****		**1,00****		2,00		**1,00****	
3	Ease of handling	2,00		2,00		**1,00*****		**1,00****		2,00		**1,00****	
4	Muscular tonus	0,00		0,00		**´-1,00****		0,00		0,00		0,00	
5	Lacrimation	0,00		0,00		0,00		0,00		0,00		0,00	
6	Palpebral closure	1,00		1,00		1,00		1,00		1,00		1,00	
7	Endo/exophtalmus	0,00		0,00		0,00		0,00		0,00		0,00	
8	Fur abnormalities	0,00		0,00		0,00		0,00		0,00		0,00	
9	Skin abnormalities	0,00		0,00		0,00		0,00		0,00		0,00	
10	Salivation	0,00		0,00		0,00		0,00		0,00		0,00	
11	Nose secretion	0,00		0,00		0,00		0,00		0,00		**3,00****	
12	Rearing	7,63	4,93	3,25	3,45	**0,88*****	**1,13**	**0,25*****	**0,46**	**1,38****	**2,50**	**1,13*****	**1,25**
13	Urination	2,75	5,12	3,13	7,66	2,71	4,15	1,13	1,64	**0,25*****	**0,46**	**0,17*****	**0,41**
14	Defecation	0,00		0,00		0,00		0,00		0,00		0,00	
15	Hyperkinesis	0,00		0,00		**3,00****		0,00		0,00		0,00	
16	Tremors	0,00		0,00		**3,00****		0,00		0,00		0,00	
17	Clonic movements	0,00		0,00		0,00		0,00		0,00		0,00	
18	Tonic movements	0,00		0,00		0,00		0,00		0,00		0,00	
19	Gait	0,00		0,00		**1,00*****		**1,00*****		**1,00*****		**1,00*****	
20	Ataxia	0,00		0,00		**1,00*****		**1,00*****		**1,00*****		**1,00*****	
21	Gait score	1,00		1,00		**2,00*****		**2,00*****		**2,00*****		**2,00*****	
22	Mobility score	1,00		1,00		1,00		1,00		1,00		1,00	
23	Arousal (GCS)	0,00		0,00		0,00		0,00		0,00		0,00	
24	Activity	4,00		**2,00****		**1,00*****		**1,00*****		**1,00*****		**1,00*****	
25	Tension	0,00		0,00		0,00		0,00		0,00		0,00	
26	Vocalisation	0,00		0,00		0,00		0,00		0,00		0,00	
27	Stereotypy	0,00		0,00		0,00		0,00		0,00		0,00	
28	Bizzare behaviour	0,00		0,00		0,00		0,00		0,00		0,00	
29	Approach response	2,00		**1,00***		2,00		2,00		**1,00***		**1,00***	
30	Touch response	2,00		2,00		2,00		2,00		2,00		**1,00***	
31	Click response	2,00		2,00		2,00		**3,00****		**3,00****		2,00	
32	Tail-pinch response	2,00		2,00		2,00		2,00		2,00		2,00	
33	Pupil size	0,00		**´-2,00*****		**´-2,00*****		**1,00*****		**´-2,00*****		**´-2,00*****	
34	Pupil response	1,00		**0,00*****		**0,5*****		**0,5*****		**0,5*****		**0,5*****	
35	RRF	1,00		1,00		1,00		1,00		1,00		1,00	
36	RRV	1,00		1,00		1,00		1,00		1,00		1,00	
37	Landing foot splay (mm)	96,94	16,58	80,75	17,97	**66,50***	30,40	81,25	18,22	86,75	15,32	65,19	44,28
38	Forelimb grip strength (kg)	6,88	0,70	**5,46****	0,76	**5,14****	0,99	**4,99***	1,91	**6,16****	0,51	**5,55****	0,29
39	Handlimb grip strength (kg)	1,03	0,21	0,94	0,16	**0,73***	0,17	0,83	0,17	0,75	0,23	**0,75****	0,14
40	Grip strength of all limbs (kg)	17,09	1,60	16,78	2,39	**12,33****	2,37	**11,39****	3,58	**15,05****	1,24	**14,23****	1,63
41	Food receiving (%)	100,00		**36,00*****		**37,14*****		**37,00*****		**42,00*****		**31,83*****	
42	Body weight (g)	207,00	20,93	202,13	12,98	205,14	43,27	207,75	12,53	193,63	16,89	194,00	14,25
43	Body temperature	37,26	0,16	**36,08****	0,52	**36,65****	0,47	**36,34****	0,52	**36,44****	0,58	**36,50****	0,35
44	Respiration	0,00		0,00		0,00		0,00		0,00		0,00	
45	Vertical activity	291,13	102,97	**47,25*****	120,06	**67,29*****	77,47	**45,75*****	103,83	**13,63****	11,94	**85,67*****	108,71
46	Horizontal activity	45,63	22,35	**5,25*****	13,66	**7,14*****	11,13	**2,25*****	6,36	**0,38*****	1,06	**8,00*****	14,41
47	Total motor activity	336,75	115,32	**52,50*****	133,69	**65,13*****	85,13	**48,00*****	110,17	**14,00*****	12,49	**70,25*****	112,35
		n= 8		n= 8		n= 7		n= 8		n= 8		n= 6	

Table 2 The values of tabun-induced neurotoxic markers measured at 7 days following tabun challenge by the Functional observational battery (No 1-11, 15-36, 44 - scored values, No 12-14, 37-43, 45-47 - values in absolute units). Statistical significance and abbreviations – see Table 2.

7 days		Controls		Tabun+A+K 203		Tabun+A+K206		Tabun+A+Hl-6		Tabun+A+obidoxime		Tabun	
No.	Marker	x/M	±s	x/M	±s	x/M	±s	x/M	±s	x/M	±s	x/M	±s
1	Posture	1,00		1,00		1,00		1,00		**3,00*****		**3,00*****	
2	Catch difficulty	2,00		2,00		2,00		2,00		2,00		2,00	
3	Ease of handling	2,00		2,00		2,00		2,00		2,00		2,00	
4	Muscular tonus	0,00		0,00		0,00		0,00		0,00		0,00	
5	Lacrimation	0,00		0,00		0,00		0,00		0,00		0,00	
6	Palpebral closure	1,00		1,00		1,00		1,00		1,00		1,00	
7	Endo/exophtalmus	0,00		0,00		0,00		0,00		0,00		0,00	
8	Fur abnormalities	0,00		0,00		0,00		0,00		0,00		0,00	
9	Skin abnormalities	0,00		0,00		0,00		0,00		0,00		0,00	
10	Salivation	0,00		0,00		0,00		0,00		0,00		0,00	
11	Nose secretion	0,00		0,00		0,00		0,00		0,00		0,00	
12	Rearing	2,13	4,05	0,50	0,76	5,75	6,16	2,38	3,46	1,00	2,07	2,00	2,88
13	Urination	0,00		0,00		0,00		0,00		0,00		0,00	
14	Defecation	0,00		0,00		0,00		0,00		**2,00***	0,45	0,00	
15	Hyperkinesis	0,00		0,00		0,00		0,00		0,00		0,00	
16	Tremors	0,00		0,00		0,00		0,00		0,00		0,00	
17	Clonic movements	0,00		0,00		0,00		0,00		0,00		0,00	
18	Tonic movements	0,00		0,00		0,00		0,00		0,00		0,00	
19	Gait	0,00		0,00		0,00		0,00		0,00		0,00	
20	Ataxia	0,00		0,00		0,00		0,00		0,00		0,00	
21	Gait score	1,00		1,00		1,00		1,00		1,00		1,00	
22	Mobility score	1,00		1,00		1,00		1,00		1,00		1,00	
23	Arousal (GCS)	0,00		0,00		0,00		0,00		0,00		0,00	
24	Activity	1,00		1,00		**5,00*****		1,00		1,00		1,00	
25	Tension	0,00		0,00		0,00		0,00		0,00		0,00	
26	Vocalisation	0,00		0,00		0,00		0,00		0,00		0,00	
27	Stereotypy	0,00		0,00		0,00		0,00		0,00		0,00	
28	Bizzare behaviour	0,00		0,00		0,00		0,00		0,00		0,00	
29	Approach response	1,00		1,00		**2,00****		1,00		1,00		1,00	
30	Touch response	2,00		2,00		2,00		2,00		2,00		2,00	
31	Click response	2,00		2,00		2,00		2,00		2,00		2,00	
32	Tail-pinch response	2,00		2,00		2,00		2,00		2,00		2,00	
33	Pupil size	0,00		**-1,00***		**1,00***		**1,00***		0,00		0,00	
34	Pupil response	1,00		**0,5*****		1,00		1,00		**0,5*****		**0,00*****	
35	RRF	1,00		1,00		1,00		1,00		1,00		1,00	
36	RRV	1,00		1,00		1,00		1,00		1,00		1,00	
37	Landing foot splay (mm)	93,31	14,87	96,94	9,94	84,25	37,96	97,25	41,02	100,13	15,21	79,13	52,22
38	Forelimb grip strength (kg)	7,03	0,91	6,28	1,57	6,17	0,60	6,26	0,58	7,04	0,63	7,50	0,49
39	Handlimb grip strength (kg)	1,30	0,18	0,94	0,17	1,19	0,16	1,30	0,13	1,31	0,20	1,15	0,23
40	Grip strength of all limbs (kg)	17,68	1,86	21,33	2,43	17,29	2,42	18,73	5,07	18,88	2,01	20,10	2,42
41	Food receiving (%)	100,00		100,00		100,00		100,00		100,00		100,00	
42	Body weight (g)	228,13	21,70	228,38	11,35	220,43	22,20	227,86	19,27	221,25	16,30	218,83	18,69
43	Body temperature	37,11	0,11	37,00	0,14	37,04	0,19	37,16	0,31	37,01	0,21	37,11	0,39
44	Respiration	0,00		0,00		0,00		0,00		0,00		0,00	
45	Vertical activity	162,63	112,56	144,88	129,28	153,14	131,55	97,14	108,45	**34,00***	83,35	**79,83***	65,10
46	Horizontal activity	14,63	11,24	31,00	35,86	25,14	25,51	10,86	19,95	7,25	20,51	10,67	9,16
47	Total motor activity	177,25	119,80	175,88	163,10	156,00	154,88	94,50	119,30	**41,25***	103,83	**67,88***	72,97
		n= 8		n= 8		n= 7		n= 7		n= 8		n= 6	

developed oxime (K206) to eliminate tabun-induced acute signs of neurotoxicity is rather low. The neuroprotective efficacy all oximes studied corresponds to their potency to reactivate tabun-inbibited AChE in peripheral as well as central compartment and to their potency to reduce acute toxicity of tabun. While the oxime K203 is markedly more effective in reactivation of tabun-inhibited AChE in rats, especially in brain, and in reducing lethal toxic effects of tabun in mice than currently available oximes [16], the oxime K206 does not prevail therapeutic and reactivating effectiveness of currently available obidoxime and trimedoxime [17]. According to our results, not only the position of the oxime group at the pyridinium ring but also the position of carbamoyl group at the pyridinium ring can play an important role in the potency of oximes to counteract acute toxicity of tabun [7,15].

Till now, there is not any broad spectrum oxime able to satisfactorily counteract acute toxic effects of all nerve agents regardless of their chemical structure [14,18]. The oxime HI-6 is the most efficacious oxime to reactivate soman or cyclosarin-inhibited AChE and protect soman or cyclosarin-exposed mammals from acute toxic effects [11,19]. Nevertheless, it is not efficacious to protect tabun-exposed animals from tabun-induced neurotoxicity [10]. Obidoxime is a suitable oxime for the reactivation of sarin or VX-inhibited AChE [18] but it is not able to sufficiently protect soman or cyclosarin-exposed mammals from their acute toxic effects [11,19]. The newly developed oxime K203 can be considered to be promising oxime for the antidotal treatment of acute tabun poisonings because its neuroprotective potency is markedly higher compared to other oximes studied.

Acknowledgements

The authors would like to thank to Mrs. E. Reslova and Mrs. J. Uhlirova for their skilful technical assistance. The study was supported by the grants of Ministry of Defence, No FVZMO0000501 and OPUOFVZ200603.

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c/o Chiba Institute of Science
15-8 Shiomi-cho, Choshi, Chiba 288-0025, Japan

Printed in Japan
