
Résumé

ON THE ANTIBACTERIAL
ACTIVITY OF PYRONE
DERIVATIVES *IN VITRO*

Studies on the Chemotherapeutic Agents. I

TADAO TODA, TÔRU TOKUNAGA, TSUTOMU
OUCHIDA, KENICHI SHIROYA & TAKAO
NAKAMOTODept. of Bacteriology, School of Medicine.
Kyushu Univ., Fukuoka, Japan

In order to investigate the relationship between chemical structure and antibacterial activity, 30 pyrone-derivatives, dehydroacetic acid type and 3-acyl-4-hydroxy coumarin type compounds, were examined.

Serial dilutions of each compounds were prepared with the media, and the minimal concentration of growth inhibition of bacteria was estimated. The bacterial strains used were *Staph. aureus* 209P, *Bac. subtilis*, *E. coli*, *Sh. flexn.* 2a, *Myc. tbc.* H37Rv and 6 *Candida* strains. The media used were KIRCHNER's 10% serum media for *Myc. tbc.*, SABOURAUD bouillon against *Candida* and nutrient broth for other bacteria.

The results showed that the pyron derivatives had the antibacterial effect against Gram positive bacteria but not against Gram negatives. In order to get the powerful activity, the compounds must have "tricarbonyl methane" type. As a rule the antibacterial power of 4-hydroxycoumarin type was higher than that of triacetic acid lactone type. Pyrone derivatives having the 3-position acyl group showed various grades of antibacterial activity according to the structure of the acyl group. Among the compounds possessing various structure at the terminal end of the acyl group, the compounds possessing methyl group had the highest antibacterial power, the second was ethoxycarbonyl group and the third carboxyl group. The α -Imino and acid amide group was not found any activity.

Toxicity of Ethyl-4-hydroxy- ι -oxo-3-coumarin-decanoate and 3-n-Decanoyl-4-hydroxycoumarin, both of them had the high antibacterial power, were examined by mice abdominal injection. The result showed that the former could not kill the mice in 1,013 mg/kg, and the latter could kill in 300 mg/kg at 2 hours after injection.

ON THE ANTIBACTERIAL
ACTIVITY OF NITROFURAN
DERIVATIVES *IN VITRO*. IIStudies on the Chemotherapeutic
Agents. IITADAO TODA, ICHIZI MIFUCHI, TOMIO
KAWATA & TÔRU TOKUNAGADept. of Bacteriology, School of Medicine,
Kyushu Univ., Fukuoka, Japan

In order to discover the chemotherapeutic agents having excellent antibacterial action, and to investigate the relationship between antibacterial activity and chemical structure, the antibacterial effect of new 9 Furan and 50 Nitrofurantoin derivatives was investigated *in vitro*. Serial dilution of each compound was made with the media, and the minimal concentration of growth inhibition of bacteria was estimated. The bacterial strains used were *Staph. aureus* 209P, *E. coli* B, *S. typhi* H901, *Sh. flex.* 2a, *Pseud. aeruginosa*, *Vibrio cholerae*-INABA and *Myc. tbc.* H37Rv. The media used were KIRCHNER's serum medium against *Myc. tbc.*, and nutrient broth against other strains.

The results showed that ;

1) Furan derivatives are not effective against all strains at the concentration of 1 : 10,000.

2) Nitrofurantoin derivatives having a 5-position nitro radical have various grades of antibacterial power against all strains without *Pseudomonas aeruginosa* according to the character of the 2-position side chain.

3) Comparison between 2-(5-Nitro)-furyl acrolein and 2-(5-nitro) furfurylidene shows that the former is more effective than the latter in general.

4) In β -(5-nitro-2-furyl) acrolein derivatives, comparison between α -methyl, α -ethyl and α -bromo radical shows that α -methyl type is the highest bacteriostatic effect against *Myc. tbc.*, and α -bromo types that against other strains.

5) Many compounds in nitrofurantoin derivatives show the excellent antibacterial activity *in vitro*. Especially β -(5-nitro-furyl)- α -methyl acrolein semicarbazone inhibits the growth of *Myc. tbc.* at 1 : 1,280,000 dilution as well as streptomycin does. And β -(5-nitro-2-furyl)- α -bromoacrolein semicarbazone, 2-(5-nitro)-furyl acrylidene picolinoyl hydrazine and 1-[2-(5-nitro)-furyl acrylidene]-2-(6-amino-3-pyridoyl)-hydrazine inhibit the growth of *St. aureus* or *V. cholerae* at 1 : 1,280,000.

STUDIES ON CANCER CHEMOTHERAPY (1 ST REPORT)

Studies on the anti-neoplastic action of MH
(Haematoporphyrin-Hg) upon EHRlich-carcinoma

NOBORU IJIMA, KIYOSHI MATSUURA,
KICHISHIRO FUJITA, TATSUO AIBA,
JINYA UKISHIMA & KEIICHI KAMISHIRADO
IInd Sugical Clinic, Medical Department,
Tokyo University

We studied the anti-tumor effect of MH upon
EHRlich solid tumor. The mechanisms were discussed especially about its activity on liver-catalase.

BACTERIOLOGICAL STUDIES ON TUBERCLE BACILLI IN THE SPECIMENS, RESECTED SURGICALLY FROM PULMONARY TUBERCULOSIS. REPORT I

Mainly in Regard with Staining and Culture

MUNEO YOKOYAMA, M. D.
Surgical Clinic, School of Medicine,
Keio University
(Director : Prof. N. SHIMADA)
Clinical Bacteriologic Institute, School
of Medicine, Keio University
(Director : Prof. D. USHIBA)

Bacteriologic investigations were carried out on 284 surgical lesions removed by segmental, subsegmental or partial resections from 204 patients with pulmonary tuberculosis, mainly localized in a segment or smaller area. The following conclusions were obtained.

1) Microscopical acid-fast bacilli and culturable bacilli were found in 172 specimens (60.5%) and 79 specimens (28%) respectively. There were much more demonstrable bacilli in cavities than in any other kind of specimens. Bacilli visible in acid-fast staining, but not culturable, remained in 115 lesions (40%), and there was no difference in the appearance of these bacilli between cavities and encapsulated foci.

2) The larger the resected lesions, the higher was the percentage of acid-fast bacilli found, but there was no correlation between the size of lesions and rate of positive culture.

3) The maximum effective duration of preoperative chemotherapy was to be between 6 and 12 months. The microscopically acid-fast-positive but not

culturable bacilli were found in high percentage in the specimens resected during this period.

4) The longer the preoperative negative period in sputum-culture, the lower the rate of bacilli demonstrable in the resected specimens.

5) There was no relationship between the number of acid-fast bacilli in the specimen and the rate of positive culture.

6) Guinea pig inoculation was more favorable method than culture for demonstration of viable bacilli.

7) It could be said that several methods of pre-treating the specimen before culture had no influence in the result.

8) Simple washing of the specimen by albumin solution did not influence the result.

9) Cultures of 210 specimens were observed in both OGAWA's egg media and fluid media, and demonstrable microorganisms grew macroscopically in just 11 specimens in fluid media, not in solid media. It is assumed that these bacilli in 10 out of 11 specimens were able to begin active growth only in the fluid culture.

To summarize, it can be suggested that there were dormant tubercle bacilli different from normal bacilli *in vitro*, which were modified into a kind of life cycle in the tuberculosis lesions under specific circumstances.

BACTERIOLOGIC STUDIES ON TUBERCLE BACILLI IN THE SPECIMENS, RESECTED SURGICALLY FROM PULMONARY TUBERCULOSIS. REPORT II

Studies on Virulence of Bacilli, which were
Culturable by Fluid Media, but not
Culturable by Solid Media

MUNEO YOKOYAMA, M. D.
Surgical Clinic, School of Medicine,
Keio University
(Director : Prof. N. SHIMADA)
Clinical Bacteriologic Institute, School of Medicine,
Keio University
(Director : Prof. D. USHIBA)

It was reported in the previous article that there were tubercle bacilli, in few specimens, resected from pulmonary tuberculosis surgically, which were unable to grow in OGAWA's egg media, but which could begin formation of colonies in the same media after culture in the fluid media for a period of 10, 30 or 60 days.

This report deals with experimental studies on the

virulence of microorganisms in 4 of the above specimens (No. 219 (1), No. 219 (2), No. 243 and No. 291), cultured by fluid media, using H37Rv strain as control.

Each strain was cultured for 2 weeks, and those cells of exact weight of 0.1 mg in each strain, diluted by 0.5% gelatin physiologic solution, were inoculated into 70 mice intravenously.

Every mouse belonged to SM clone, male, aged 5 weeks, weighing 16~23 g (average 17.8 g). The animals were treated under constant conditions.

The number of viable cells in lung, liver, spleen and kidney, and ratio in weight of spleen to that of body weight were investigated weekly.

The results obtained were as follows.

The microorganism of 3 strains (No. 219 (1), No. 219 (2), and No. 243) were scarcely different from those of H37Rv in virulence, almost always showing the same attitude as those of H37Rv.

The microorganisms of the last strain (No. 291) had ability to multiply and grow more vigorously in mice than other strains, especially 8 weeks after inoculation.

It was demonstrated that the virulence of these bacilli of every strain was great in mice at least in these experimental studies.

INVESTIGATION OF *CANDIDA* WITH THE FLUORMICROSCOPE. PART I

Fundamental Study on the Differentiation
of Living and Dead *Candida albicans*

ICHIRO OHIRA, SAKAE OHASHI,
FUJIYA IWASAKI & TSUGIO ENDO

HAYASHI's Dept. of Internal Medicine, Tokyo
Jikei-Kai School of Medicine

Living and dead *Candida albicans* (hereafter, referred to as *C. alb.*) could be differentiated under the fluormicroscope when they were adequately stained with acridinorange (hereafter, referred to as A O).

The most suitable fluorochrome to differentiate *C. alb.* was obtained when stained with aqueous solution of A O with concentration from 1 : 8,000 to 1 : 10,000.

Living *C. alb.* stained with above concentration of A O emitted greenish fluorescence while dead ones reddish fluorescence, which was also confirmed by the culture of *C. alb.* Toxicity would be hardly considered with this concentration of A O.

The best conditions for this treatment were 0.5-1 mg/cc of the amount of *C. alb.*, 14-15°C of the treating temperature while staining and microscopic observation should be done within 15~20 minutes.

A QUANTITATIVE ANALYSIS OF THE DRUG RESISTANCE OF *MYCOBACTERIUM* *TUBERCULOSIS* IN SPUTUM. REPORT VI

Confidence Limits of the Methods of
Testing Drug Resistance

MICHIO TSUKAMURA, MASAKUNI YAMAMOTO,
MITSUO HAYASHI & EIICHI NAKAMURA

The Obuso National Sanatorium, Obu, Aichi

The number of viable units of *Mycobacterium tuberculosis* occurring in sputum was estimated on various sputa by the method described by us (the enumeration of viable *Mycobacterium tuberculosis* by egg medium slant and whirled loop inoculation, Kekkaku, in press, 1958~1957), and standard deviations in the estimations were estimated and expressed as percentage standard deviations. From these estimates, a possible maximal value of the percentage standard deviation was determined as 32% at 95% confidence rate.

Substituting this value in a given case, the following postulations have been presented, assuming that the estimation will be made under the same conditions and the number of colonies of a given estimate is not less than 10.

The mean value of viable units in sputum (\bar{x}) that should be obtained by more accurate experiments using many tubes would be between the following limits.

If x is an estimate obtained by only one tube,
at 70% confidence rate, $(x \pm 0.32x)$,
at 95% confidence rate, $(x \pm 0.64x)$.

If x is an average of two tubes,
at 70% confidence rate, $(x \pm 0.23x)$,
at 95% confidence rate, $(x \pm 0.46x)$.

If x is an average of three tubes,
at 70% confidence rate, $(x \pm 0.19x)$,
at 95% confidence rate, $(x \pm 0.37x)$.

See also table.

(Printed in Vol. 6 No. 1)

THE EFFECT OF HYDRONSAN FOR SEVERE PULMONARY TUBERCULOSIS

Chief · ROKURŌ KATSUNUMA,
KŌJI MIURA, MITSUO HAYASHI, TAKASHI
ABO & HUTORI TORII

The Obuso National Sanatorium,
Obu, Chita, Aichi

Twenty-two patients of severe pulmonary tuberculosis were given hydronsan. The medicine was given during six months, and the daily doses were from 2g to 5g. The resistance to antibiotics of *Mycobacterium tuberculosis* in sputum, the chest-roentgenphotograph, the progress of clinical symptom were observed. And as above mentioned, thirteen patients were given isoniazid. Hydronsan was manufactured by Chugai Seiyaku Co., Ltd, and sodium isonicotinyldiazide glucuronate.

1) The maximum doses was two and a quarter times as much for hydronsan as for isoniazid,

2) On the appearance of resistance to antio-

tics, the effect of antibiotics in the group of patients treated with hydronsan was equal to that in the group with isoniazid.

3) On the progress of clinical symptom and on the effect of the chemotherapy so far, the number of improved patients in the group of treated with hydronsan was greater than that in the group with isoniazid.

4) The blood concentration of drug was from 2 to 2.5 times as much in 3g of hydronsan as in 1g isoniazid.

(Printed in Vol. 6 No. 1)