INHIBITION OF DEOXYRIBONUCLEASE BY NALIDIXIC ACID, PIROMIDIC ACID AND PIPEMIDIC ACID

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SUMMARY

Deoxyribonuclease (DNAase) from calf-thymus was inhibited by three antibacterial drugs; nalidixic acid (NA), piromidic acid (PA) and pipemidic acid (PPA). The order of inhibitory activity was estimated as PPA>PA>NA. This order was similar to that of static activity of these drugs against Gram-negative bacteria. A correlation of the DNAase inhibition with the antibacterial mechanism is discussed with respect to the inhibition of DNA synthesis or DNA repair.

INTRODUCTION

It has been well investigated that three drugs; nalidixic acid (NA)[1], piromidic acid (PA)[2] and pipemidic acid (PPA)[3] have more potent static activities against Gram-negative bacteria and their actions are due to inhibition of bacterial DNA synthesis[4,5]. Though there have been many reports on these drugs, especially on NA, the exact mechanism of inhibition of DNA synthesis is not known[6]. Several current papers[7,8] showed that NA inhibited the DNA-replication and blocked the repair synthesis of DNA. However, effects on DNA polymerase have been shown to be of negative importance[6].

A preceding paper[9] from our laboratory reported that NA, PA and related compounds accelerated the electron transfer from inorganic ferrous ion (Fe^{2+}) to cytochrome c and this acceleratory activity was parallel to their antibacterial activity. This suggested that these drugs might act as an energetic uncoupler of DNA synthesis or DNA repair, owing to their interference with certain bioenergetic electron transfer.

In order to substantiate this explanation, it should be necessary to demonstrate that these drugs have a high tendency to be localized in,

tively. It is apparent that the inhibition of DNAase was dependent on the NA concentration and was remarkable at the concentration above 2×10^{-4} M.

Effects of PA and PPA at their concentrations of $2\times10^{-5}\sim2\times10^{-4}$ M on the DNAase activity are shown in Figs. 2 and 3, respectively. It is also apparent that the inhibition was concentration-dependent and that a potent inhibition is observed with PA at 8×10^{-5} M and more potent inhibition with PPA at an equimolar concentration. It is also to be noted that the inhibitions of other related compounds were less potent in spite of their structural similarity to PA and PPA, while AT-749 having a somewhat different structure showed a strong inhibition. Subsequently, the order of DNAase inhibition: PPA>AT-749, PA>AT-88 > NA>AT-593>AT-22 has been estimated.

DISCUSSION

The present study declares that three synthetic antibacterial drugs, NA, PA and PPA have potent inhibitory effects on the DNAase activity *in vitro,* and the order of their enzyme inhibition is parallel to the order of antibacterial activity, or more closely static activity against Gram-negative bacteria as shown in Table 1. Of certain interest is that only PPA, the most potent inhibitor of DNAase in this group could be static against *Pseudomonas*[14]. It is also to be noted that with few exceptions, the above parallelism observed for NA, PA and PPA can be further extended to other related compounds whose antibacterial activity is also shown in Table 1. And thus, AT-749, a relatively stronger inhibitor of DNAase shows a potent antibacterial activity, while AT-593, the least potent inhibitor is the lowest in its activity.

There are two main subjects to be discussed from the aspect of chemico-biological interactions. The one is to correlate the inhibition of DNAase by these drugs with the inhibition of bacterial growth at a biochemical level. The other is to interpret the mechanism of inhibition of DNAase by constructing a molecular model.

Of certain importance for the first subject might be the role of

or have a special affinity for the multienzyme system associated with DNA synthesis or DNA repair. The present work thus intends to examine their effects on the enzyme systems. Deoxyribonuclease (DNAase) has been utilized in view of the fact that it has recently been recognized as an important component of multienzyme system of DNA synthesis or DNA repair[10,11]

MATERIALS AND METHODS

Highly polymerized DNA preparation from calf-thymus, and crystalline DNAase from bovine pancreas were purchased from Sigma Chemical Company, U. S. A. NA was prepared by $CHCl_3$ extraction from commercial tablets. PA, PPA, and related compounds were kindly provided by Dainippon Pharmaceutical Company, Osaka, Japan. The compounds other than NA, PA and PPA were coded as follows: AT-22, AT-88, AT-593 and AT-749[12]. The structural formulas and antibacterial activity of all these compounds are listed in Table 1 of the DISCUSSION.

The rate of the enzyme reaction was measured according to Kunitz's method[13] partly modified by the author; the increase of absorbance (at 260 nm) of the system in the 10 mm-optical path cuvette was followed with a Hitachi UV-VIS spectrophotometer. The reaction mixtures contained 0.1 mg/ml DNA, 0.2×10^{-2} M $MgCl_2$, 10 μg/ml DNAase, and a drug at four different concentrations of $2\times10^{-4}\sim2\times10^{-5}$ M in 0.02 M acetate buffer at pH 7.0. In individual determinations, the initial absorbance of the system was adjusted to be 50.0% by equilization of DNA concentration in both the sample and the reference cuvette.

All measurements were carried out at 23°C.

RESULTS

Effects of NA on the DNAase activity are shown in Fig. 1. Curve a is a standard time course (control) of the DNA-DNAase system in the absence of NA, and curves b, c, d and e are time courses in the presence of NA at concentrations of 2×10^{-5}, 4×10^{-5}, 8×10^{-5} and 2×10^{-4} M, respec-

DNAase in both the DNA synthesis and the DNA repair. As summarized in reviews by LEHMAN[10], and HOWARD-FLANDERS[11], DNAase performs some accessory function in DNA replication and DNAase initiates the repair replication by excising the damaged DNA. Accordingly, it is quite reasonable to consider that the inhibition of DNAase by NA, PA and PPA could interfere with DNA synthesis and/or DNA repair, which might lead to suppression of the bacterial growth.

Of certain significance for the second subject might be the role of Mg^{2+} for the DNAase activity. As has been reported in detail by KUNITZ, Mg^{2+} is the most potent activator, which can form a DNA-complex to be susceptible to the enzyme action. Since NA, PA and PPA have been proved to form a Mg^{2+}-chelate compound[15], it is quite possible that these drugs could interfere with the DNA-Mg^{2+}-DNAase binding through chelation. In addition, it may be also an important factor that a purine-like structure of these drugs could favor a specific binding with the basophilic site of DNAase.

As Howard-Flanders indicated in his review, repair in excision, recombination and replication of DNA may proceed in the integrated enzyme complex rather than as independent reactions. If the energy yielding system involving biological electron transfer might operate in this complex, these drugs could have dual effects on the processes of DNA synthesis or repair; one might be inhibition of DNA synthesis or repair and the other bioenergetic uncoupling.

To make such a possibility of the dual effects of NA, PA and PPA more confirmatory, it may be important to verify that the DNAase extracted from the Gram-negative bacteria can be also inhibited and that another energetic uncoupler can enhance the antibacterial activity of these drugs. Some of these experiments are in progress, and will be reported later.

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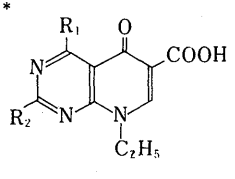
REFERENCES

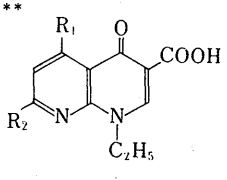
1) Lesher, G. Y., E. J. Froelich, M. D. Gruett, J. H. Bailey & R. P. Brundage: 1, 8-Naphthyridine derivatives: a new class of chemotherapeutic agents. J. Med. Pharm. Chem. *5,* 1063~1065, 1962
2) Minami, S., T. Shono & J. Matsumoto: Pyrido [2, 3-d] pyrimidine antibacterial agents II. Piromidic acid and related compounds. Chem. Pharm. Bull. (Tokyo) *19,* 1426~1432, 1971
3) Matsumoto, J. & S. Minami: Pyrido [2, 3-d] pyrimidine antibacterial agents. III. 8-Alkyl- and 8-vinyl-5, 8-dihydro-5-oxo-2-(1-piperazinyl) pyrido [2, 3-d] pyrimidine-6-carboxylic acids and their derivatives. J. Med. Chem. *18,* 74~79, 1975
4) Goss, W. A., W. H. Deitz & T. M. Cook: Mechanism of action of nalidixic acid on *Escherichia coli.* J. Bact. *88,* 1112~1118, & 1068~1074, 1964
5) Nakamura, S., M. Ishiyama & M. Shimizu: Mechanism of action of piromidic acid. Chemotherapy *21,* 1885~1886, 1973
6) Gale, E. F., E. Cundliffe, P. E. Reynolds, M. H. Richmond & M. J. Waring: "The Molecular Basis of Antibiotic Action", John Wiley & Sons, London, Pp. 264~267, 1972
7) Ramareddy, G. & H. Reiter: Specific loss of newly replicated deoxyribonucleic acid in nalidixic acid-treated *Bacillus subtilis* 168. J. Bact. *100,* 724~729, 1969
8) Ward, C. B., M. W. Hane & D. A. Glaser: Synchronous reinitiation of chromosome replication in *E. coli* B/r after nalidixic acid treatment. Proc. Natl. Acad. Sci. *66,* 365~369, 1970
9) Yamabe, S.: Acceleratory effects of nalidixic acid and piromidic acid on the electron transfer from $FeSO_4$ to cytochrome c. J. Antimicr. Chemoth. in submission.
10) Lehman, I. R.: Deoxyribonucleases: Their relationship to deoxyribonucleic acid synthesis. Ann. Rev. Biochem. *36,* 645~668, 1967
11) Howard-Flanders, P.: DNA repair. Ann. Rev. Biochem. *37,* 175~200, 1968

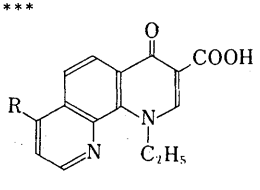
12) Minami, S., Y. Takase & S. Yamabe: Synthesis of 1, 10-phenanthroline-8-carboxylic acid derivatives. Japan. Patent, 1971
13) Kunitz, M.: Crystalline Deoxylibonuclease. II. Digestion of thymus nucleic acid. The kinetics of the reaction. J. Gen. Physiol. *33*, 363~377, 1950
14) Shimizu, M., Y. Takase, S. Nakamura, H. Katae, A. Minami, K. Nakata, S. Inoue, M. Ishiyama & Y. Kubo: Pipemidic acid, a new antibacterial agent active against *Pseudomonas aeruginosa: in vitro* properties. Antimicr. Agents & Chemoth. 132~138, 1975
15) Yamabe, S.: unpublished data

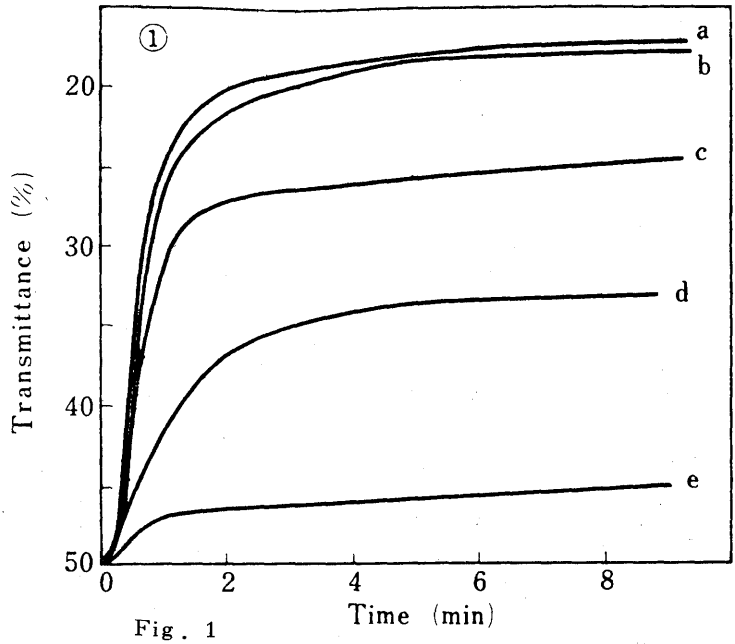
TABLE 1 *IN VITRO* ANTIBACTERIAL ACTIVITY OF NA, PA, PPA AND RELATED COMPOUNDS.

Test bacteria	*Antibacterial activity* (MIC, μg/ml)						
	AT-22	AT-593	AT-88	NA	PA	AT-749	PPA
Staph. aureus	>100	>30	30	100	10	3	30
E. coil	>100	30	3	1	1	1	1
Shig. flexneri	>100	30	10	3	10	3	1
Pseud. aeruginosa	>100	>30	>100	100	100	>30	10
Chemical formulas	* R_1 : CH_3 R_2 : CH_3	* R_1 : H R_2 : CH_3	* R_1 : H R_2 : 	** R_1 : H R_2 : CH_3	* R_1 : H R_2 : 	*** R : Cl	* R_1 : H R_2 :

* 

** 

*** 


①
a
b
c
d
e
20
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50
0
2
4
6
8
Transmittance (%)
Time (min)

Fig. 1

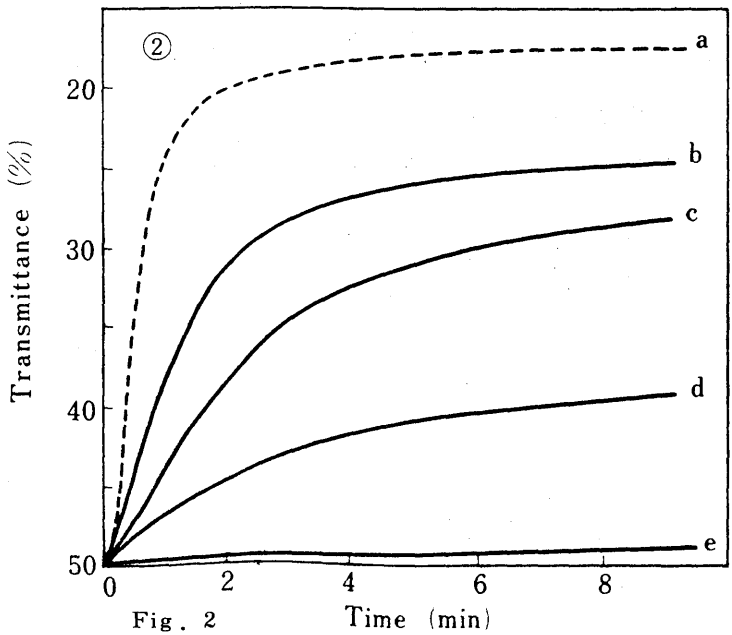
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Fig. 2

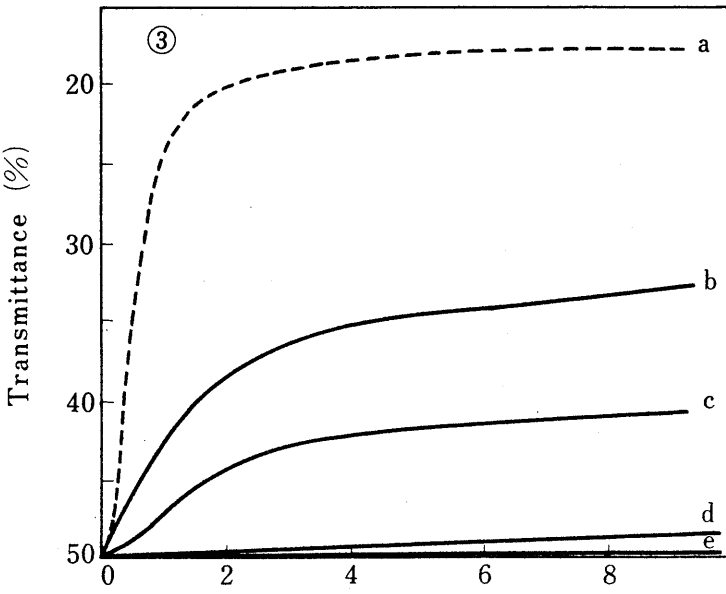

Fig. 3

LEGENDS TO FIGURES

Fig. 1. Effects of different concentrations of NA on DNAase activity
a: control (no NA), b: 2×10^{-5} M NA, c: 4×10^{-5}M NA,
d: 8×10^{-5} M NA, e: 2×10^{-4} M NA

Fig. 2. Effects of different concentrations of PA on DNAase activity
a: control, b: 2×10^{-5} M PA, c: 4×10^{-5} M PA,
d: 8×10^{-5} M PA, e: 2×10^{-4} M PA

Fig. 3. Effects of different concentrations of PPA on DNAase activity
a: control, b: 2×10^{-5} M PPA, c: 4×10^{-5} M PPA,
d: 8×10^{-5} M PPA, e: 2×10^{-4} M PPA