Arch. Pharm. Res. **14**(1), 73-77 (1991)

Effect of Pyridoxine on Rifampicin Toxicity

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(Received September 16, 1990)

Abstract □ The effects of pyridoxine (PN) on rifampicin (RMF) toxicity were investigated by both *in vivo* and *in vitro* methods. RMF (30 mg/kg) was injected intraperitoneally and PN(150 mg/kg) was administered orally to rats for 10 consecutive days. After treatment, clinical chemistry and hematologic profiles were measured. RMF and PN plus RMF did not show any adverse effects at this *in vivo* experimental condition. Thymidine incorporations of mice bone marrow cells were examined *in vitro*. RMF showed a decrease in thymidine uptake in a dose-dependent manner, but PN showed a reversal of the thymidine uptake suppression caused by RMF ($p<0.01$). On the other hand, PN showed a decrease in thymidine uptake at a high concentration level.

Keywords □ Pyridoxine, rifampicin, clinical chemistry, hematologic profile, thymidine incorporation.

Vitamin B_6 exists in three forms-pyridoxine, pyridoxamine and pyridoxal. These forms of vitamin B_6 are widely distributed in animal and plant sources; cereal grains are an especially rich source. After ingestion, vitamin B_6 is first converted to pyridoxine phosphate and then to pyridoxal phosphate (PLP), the conversion being activated by a kinase and an oxidase, respectively[1]. The conversion of pyridoxine to PLP, taking place mainly in the liver, requires both time and intact enzyme system[2]. PLP, the active cofactor form of vitamin B_6, is a coenzyme that participates in the catalysis of several important reactions of amino acid metabolism known as transamination, decarboxylation, and racemization. PLP is also required for enzyme catalysed reaction of glycogen metabolism, and porphyrine synthesis, in which it participates through formation of Schiff base. PLP readily forms covalent complexes with a variety of drugs, metabolites and toxicants[3]. PLP has been demonstrated to form complex with cyanide, gentamycin, and endogeneously generated polyamine spermidine both *in vivo* and *in vitro*[4]. The covalent complexes range from Schiff bases and reduced or cyclized Schiff bases to cyanohydrins and hydrazones. For many of these substances, pyridoxine is an effective antidote; e.g., isoniazid and hydrazine[5].

We sought to investigate the action of pyridoxine · HCl (PN) as an antidote for toxicity of rifampicin (RMF). RMF is a broad spectrum antibiotics widely employed against tuberculosis and other baterial infections[6]. Long-term administration of RMF has been shown to shorten the biological half-life of several drugs and RMF itself, presumably through induced alterations in hepatic metabolism[7]. RMF may induce abnormalities in hepatic function, a toxic effect which may progress to necrosis[8]. Eucaryotic cells *in vitro* are sensitive to RMF in concentration of 20-150 μg/ml, depending on the cell systems and on the condition of growth[9]. There is an evidence that multiply cells are more sensitive than resting ones[10]. The molecular mechanism of the toxic effect on eucaryotic cells is not understood. Since antituberculosis drugs and PN are used simultaneously in tuberculosis therapy, we have been interested in the antidotal effects its mechanism of PN on the toxicity of RMF. Thus, we have studied to elucidate the antidotic effects and mechanism of PN on the RMF toxicity using both *in vivo* and *in vitro* methods.

MATERIALS AND METHODS

Animal treatment

Male Sprague-Dawley rats (160-180 g) were used

Table I. Body weight gain and relative organ weights in rats.

	Body weight gain (g)	Liver/body weight ratio ($\times 10^{-3}$)	Kidney/body weight ratio ($\times 10^{-3}$)	Spleen/body weight ratio ($\times 10^{-3}$)	Thymus/body weight ratio ($\times 10^{-3}$)
Control	51.0±9.2	39.2±2.0	9.5±0.5	3.2±0.6	2.5±0.4
RMF	52.7±8.2	38.8±1.8	9.4±0.4	2.9±0.2	2.2±0.8
RMF+PN	51.3±7.4	41.6±1.9	9.7±0.3	3.2±0.7	2.6±0.3

Data are expressed as mean±S.D.

Table II. Hematological profile in rats

	WBC ($\times 10^3$/mm^3)	RBC ($\times 10^6$/mm^3)	Hb (g/dl)	Ht (%)
Control	5.90±1.53	4.88±0.02	13.8±0.34	42.6±1.34
RMF	6.56±2.74	5.01±0.12	14.3±0.42	44.7±7.41
RMF+PN	6.64±2.42	4.94±0.06	13.9±0.35	42.8±1.47

Data are presented as mean±S.D.
WBC, White blood cell; RBC, Red blood cell; Hb, Hemoglobin; Ht, Hematocrit.

in the *in vivo* study and inbred male CBA mice (6 weeks old) were used in the *in vitro* experiment. They were fed commercial rodent chow and water *ad libitum*. The animals were housed at the temperature of 22℃ with 55% relative humidity and controlled 12 hr cycle. Rifampicin (supplied by Dong-A Pharm. Co., Seoul) was dissolved in 10% dimethylformamide and then diluted with sterilized saline. The drug was given intraperitoneally for 10 consecutive days at a daily dose of 30 mg/kg. Pyridoxine · HCl (Sigma Chemical Co., St. Louis) was dissolved in sterilized saline and was administered orally at a daily dose of 150 mg/kg. The dosages of the drugs were calculated from human therapeutic dosages. The control group received equal volume of sterilized saline. In the *in vitro* experiment, RMF and PN were dissolved in dimethylsulfoxide and PBSS, respectively. After treatment, blood was collected by cardiac puncture and some of them (1.5 ml) were used for hematological parameter measurement and others were used for serum preparation.

Measurement of hematological and serological parameter

White blood cell (WBC) count was enumerated by microscopy method[11]. Blood was drawn to the 0.5 mark of white blood cell diluting pipette and then the Turk diluting fluid (Green Cross, Seoul) was drawn to the mark 11. The number of leukocyte was determined in a Hemacytometer. Red blood cell (RBC) count was enumerated by phase microscopy method[11] Blood was drawn to the 0.5 mark of red blood cell diluting pipette and then the Gower solution (Green Cross, Seoul) was drawn to the mark 101. The number of erythrocyte was determined in a Hamacytometer. Hematocrit was determined by microhematocrit method[11]. Blood was filled in heparinized capillary and centrifuged at 8,000×g for 2 min at room temperature. Serum/plasma ratio was calculated as a hematocrit. Hemoglobin was determined by the cyanomethohemoglobin method[11]. Blood (20 μl) was mixed with 5 ml of Drabkin solution (Green Cross, Seoul), and then the hemoglobin was determined with a Hemoglobinmeter (LK 540:Lars Ljunberg, Sweden). The hemoglobin values were determined at the wavelength of 540 nm, using cyanomethohemoglobin as a standard. The percentages of lymphocyte, monocyte and granulocyte were determined by Wright-staining method. Total protein concentration and albumin were determined by Bromocresol-green method. Precinom U(Boeringer Mannheim, Germany) was used as a standard.

Thymidine incorporation of mice bone marrow

Mice were killed by cervical dislocation and femus removed asceptically. The bone ends were punctured with a 25 gauge needle. The marrow was expelled by pushing PBSS through the center of the bones

to obtain a single cell suspension. After centrifugation (1,000×g, 5 min), the supernatant was decanted and the cells were reconstituted in 1 ml of PBSS. The cell viability was determined by trypane blue exclusion and the concentration was adjusted to 6×10^6 cells/ml of PBSS. DNA synthesis of bone marrow cell cultures was determined by the addition of 1 μ Ci of ^{3}H-thymidine (New England Nuclear, Boston, U.S.A.) to each culture. The culture consisted of 100 μl of cell suspension and an equal volume of drug sample. After CO_2 incubation (37℃, 1 hr), the cells were harvested on the filter using Titertek havester. The filters were taken up in a 5 ml toluene and counted in a beta scintillation counter (Model 5385).

Statistics

Significant difference between two groups (RMF treated group vs. RMF plus PN treated group) was determined by Students t-test

RESULTS AND DISCUSSION

In the *in vivo* study, body weight gain and relative organ weight (liver, kidney, spleen, thymus) did not show any significant changes compared to control group (Table I). In addition, there were no significant differences in the hematological profiles (WBC, RBC, Hb, Ht) and serum proteins (total protein, albumin, globulin) between those groups (Table II, IV). Lymphocyte ratio was significantly increased and granulocyte ratio decreased but monocyte ratio was not changed by PN treatment. In the *in vitro* study, rifampicin showed a decrease in thymidine uptake in a dose-dependent manner (Fig. 1) and pyridoxine showed a decrease in thymidine uptake at high concentration above 10 mM (Fig. 2). On the other hand, pyridoxine showed a reversal of the thymidine uptake suppression caused by RMF ($p<0.01$) (Fig. 3).

The adverse reactions to rifampicin occur infrequently. There are a number of reports that rifampicin causes immunosuppression in both humans and laboratory animals. However, because of the lack of uniformity in assessing immunosuppression by rifampicin, a lot of the existing data are conflicting. In humans, serum immunoglobulin concentration and serum antibody titers after immunization have been variously reported to be normal or slightly depressed, the tuberculin delayed hypersensitivity skin reaction to be increased, unaffected or depressed. It has been reported that the mean total leukocyte count was higher in patients treated with rifampicin, but this increase was accompanied by a fall in the percentage of lymphocytes. And it has been reported that the examination of albumin concentration, and the serum globulin concentration showed no suggestion of any difference during or after chemotherapy between the patients treated with rifampicin and those not treated, nor were there differences between the control subjects receiving rifampicin and those who were given only placebo[12]. Forsgren *et al.*[13,14] found that rifampicin inhibits chemotaxis of human polymorphonuclear leukocytes *in vitro* and ascribed the effect of inhibition of protein synthesis necessary for chemotaxis of the leukocytes. RMF showed no significant adverse effects in this *in vivo* experimental condition (human therapeutic dosage), so we could not elucidate the effect of PN on RMF toxicity. It has been found that stimulation *in vitro* by phytohemaglutinin or purified protein derivative tuberculosis significantly

Table III. Differential leukocytes in rats

	Lymphocytes (%)	Monocytes (%)	Granulocytes (%)
Control	85.7±6.7	2.2±1.2	13.2±3.0
RMF	78.6±3.9	1.8±1.4	19.6±4.8
RMF+PN	86.2±4.4*	1.8±0.8	12.0±2.5

Data are presented as mean±S.D.
*represent significant differences from RMF treatment alone ($p<0.05$).

Table IV. Serum profile in rats

	Trotal protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control	5.74±0.54	3.88±0.68	1.86±0.76	2.12±1.47
RMF	5.40±0.45	3.45±0.28	1.83±0.65	1.89±0.75
RMF+PN	5.82±0.73	3.88±0.46	1.93±0.49	2.01±0.38

Data are expressed as mean±S.D.

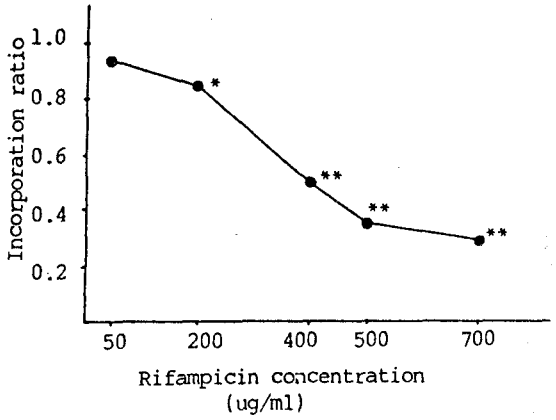

Fig. 1. Ratio of ^{3}H-thymidine incorporation in various rifampicin concentration compared to control.
Each value is expressed as the mean±S.D. of incorporation ratio.
*p<0.05, **p<0.01.

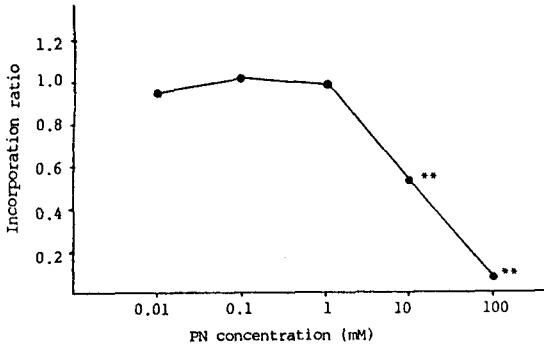

Fig. 2. ^{3}H-Thymidine uptake ratio of bone marrow cell cultured with PN.
Results were expressed as incorporation ratio compared to control.
Each value is expressed as the mean±S.D. of incorporation ratio.
**p<0.01.

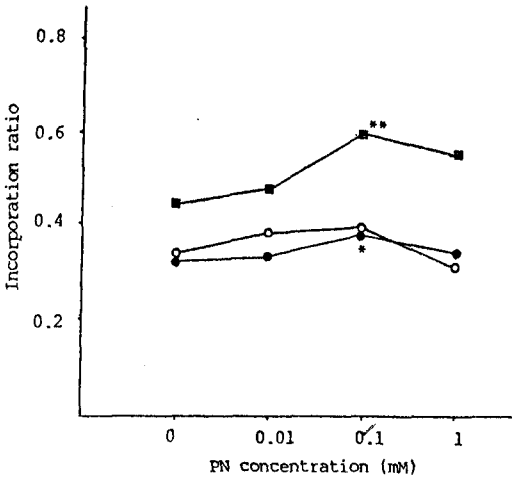

Fig. 3. Influence of PN on thymidine uptake suppression caused by rifampicin.
Results were expressed as incorporation ratio of PN supplemented cultures compared with cultures supplemented only rifampicin.
Each value is expressed as the mean±S.D. of incorporation ratio.
*p<0.05, **p<0.01
@: Cultures supplemented only rifampicin.
■-■ RMF 400
○-○ RMF 500
●-● RMF 700

inhibited at the concentration of 50-100 μg/ml[15]. It has been reported that rifampicin can suppress *in vitro* conversion of lymphocytes to lymphoblasts and uptake of thymidine[10,15,16]. In this experiment, RMF showed cell toxicity at the concentration of above 200 μg/ml. Cell line employed and incubation time contribute to the difference in RMF concentration.

PN is needed in protein and nucleic acid synthesis [17]. Cells cultured in the absense of PN showed no cell growth and in fact were dying. Cell growth was optimal at concentration of 0.005 mM. Cell growth slowed above this concentration, and cell death occurred at the concentration of 5 to 10 mM[18]. It has been demonstrated that when added to tissue culture medium at a high dosage PN prevents the proliferation of Fu5-5 rat hepatoma cells.

In this study, rifampicin showed a decrease in the thymidine uptake in a dose dependent manner and pyridoxine showed a decrease in thymidine uptake at a high concentration above 10 mM. But pyridoxine showed a reversal of the thymidine uptake suppression caused by RMF at the lower concentration ($p<0.01$). The precise mechanism by which PN inhibits the uptake of thymidine and the antidote effect of pyridoxine on rifampicin need further study.

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