Effect of Rifampin on the Immune Response in Guinea Pigs

BASEM M. DAJANI, JOHN E. KASIK, AND JOHN S. THOMPSON

Department of Internal Medicine, University of Iowa Hospital, and the Medical Service, Veterans Administration Hospital, Iowa City, Iowa 52240

Received for publication 13 October 1972

The effect of an oral dose of 20 mg of rifampin per kg per day on the immune response to killed *Mycobacterium bovis* var. BCG, 1-chloro-2,4-dinitrobenzene (DNCB), and sheep red blood cells (SRBC) was studied in adult female guinea pigs. Fully established cutaneous reactions to BCG or DNCB were markedly reduced during rifampin treatment but reverted to pretreatment levels after the drug was discontinued. When treatment was begun 7 days after immunization, positive reactions did not occur during drug administration but developed after cessation. When treatment was initiated 7 days before immunization, the response to these agents was completely suppressed during but also after rifampin was discontinued, suggesting that recognition and processing of the immunogens had been impaired. Rifampin had no effect on the secondary response to SRBC. When treatment was initiated either 7 days before or 2, 3, or 4 days after SRBC immunization, the primary response was abolished, but the reaction to a booster after drug withdrawal varied: some animals developed a normal secondary hemagglutinin response, but the majority reacted as if they had not been previously immunized. These studies indicate that rifampin is an active immunosuppressive agent in the guinea pig.

Rifampin, an orally active 3-(4-methylpiperazynyl)-iminomethyl derivative of rifamycin SV, is now widely used for the treatment of certain forms of human pulmonary tuberculosis (3, 6) and carriers of *Neisseria meningococcus* (2). Toxic manifestations including reversible leukopenia, hyperbilirubinemia, and elevation of hepatic enzymes have been reported infrequently in patients administered the recommended dosages (3, 6, 12).

Paunescu (5), however, has demonstrated suppression of the primary antibody and delayed skin test response to bovine serum albumin (BSA) in rabbits and guinea pigs receiving rifampin. Nilsson (4) reported that the drug impaired in vitro human lymphocyte stimulation by phytohemagglutinin (PHA) and purified protein derivative (PPD), but we found that suppression of PHA stimulation occurred only when the concentration exceeded 100 µg/ml of culture fluid (1). We have observed a progressive reduction in the skin test reaction to stabilized PPD-S in 14 patients with active pulmonary tuberculosis during treatment with rifampin which is reversible upon cessation of

therapy (Mukerjee and Kasik, submitted for publication). Very recently, Serrou et al. reported that rifampin prolonged the survival of skin allografts in rabbits and pancreatic grafts in dogs (8).

Taken together, these preliminary observations suggest that rifampin has immunosuppressive properties, a characteristic that could have broad implications with respect to its use in debilitated patients with chronic active tuberculosis. Therefore, the present studies were undertaken to investigate the effects of a dosage employed clinically (7, 12) on primary secondary, and established immune responses in guinea pigs immunized with sheep red blood cells (SRBC), *Mycobacterium bovis* var. BCG (BCG), and 1-chloro-2,4-dinitrochlorobenzene (DNCB).

MATERIALS AND METHODS

Animals. One- to two-month-old, randomly bred female guinea pigs were obtained from the Loren Hoeg farm, Tracer, Iowa. Depending on the protocols listed below, groups of 20 to 35 animals were immunized with each antigen. Subgroups of five animals each were employed for each individual experiment unless otherwise stated.

Immunization. The following procedures were employed to immunize the animals.

BCG: a total volume of 0.4 ml of an emulsion containing 25 mg of 2% phenol-killed BCG organisms (Tice)/ml was prepared with equal portions of sterile saline and incomplete Freund adjuvant (Difco), and 0.1-ml portions were injected into all four foot-pads.

DNCB: animals were sensitized by applying to the ears 0.05 ml of 10% DNCB (Eastman Kodak Co., Rochester, N.Y.), in a 1:1 mixture of olive oil and acetone.

SRBC: sheep blood was obtained in Alsever solution and washed three times in sterile phosphate-buffered isotonic saline (PBS), pH 7.1; 2 ml containing 2×10^8 freshly prepared SRBC in PBS was injected intraperitoneally. The same procedure was followed for booster injections.

Detection of the immune response. Antigens were injected intradermally or painted on ether-cleaned flank skin 1 day after depilation with Nair (Carter Products, New York, N.Y.). Five units of PPD-S (Parke, Davis & Co., Chicago, Ill.) in 0.1 ml was injected intradermally, and the greatest diameter of induration was measured with a vernier caliper at 48 h. Initially, measurements were recorded at 4, 24, and 48 h. Since the responses at 4 h were quite variable and no difference was observed between the 24- and 48-h values, the latter was chosen as the single best parameter to record. A positive skin reaction was defined as one greater than 5 mm in diameter since nonimmunized animals occasionally had reactions to PPD-S in the range of 2 to 4 mm in diameter.

Hypersensitivity to DNCB was detected by painting 0.025 ml of a 0.5% solution of DNCB in a 1:1 mixture of olive oil and acetone on flank skin. The double fold of skin thickness was measured at 48 h with a vernier caliper.

Humoral response to SRBC. To detect antibodies to SRBC, we collected blood from normal and immunized animals in microhematocrit capillary tubes after nicking the foot pads with a scalpel. These tubes were sealed and centrifuged at 11,500 rpm for 30 s. Sera were harvested and stored at -20°C until tested. The anti-SRBC titers were measured by capillary hemagglutination (9). In brief, washed SRBC, serum, and buffer were mixed in microtiter plates. After incubation for 2 h at room temperature, contents of the wells were aspirated into microhematocrit capillary tubes, sealed, and centrifuged. The tubes were then placed, sealed end up, at a 45° angle for 1 h. The length of the cell streamer was measured in millimeters. Semilogarithmic linear regression curves were made, and the point of 50% migration as compared with the controls (CA_{50}) was determined as the titer.

Certain sera were also treated with 2-mercaptoethanol (2-ME) to determine the relative concentration of 2-ME-sensitive immunoglobulin M (IgM) antibody in the sample. A 1:100 dilution of serum in a final concentration of 0.1 M 2-ME was incubated for 30 min at 30°C . Titration of the serum was performed without removing 2-ME (11).

Rifampin. Rifampin (kindly supplied by the Dow Chemical Co., Indianapolis, Ind., and Ciba Pharmaceuticals, Summit, N.J.) was administered to the animals every morning by gastric intubation as one daily dose of 20 mg/kg. A 20 mg/ml suspension in distilled water was freshly prepared every 2 days.

RESULTS

Throughout the course of these studies, the guinea pigs treated with rifampin appeared healthy. No change in activity or other gross change distinguished these animals from the untreated control animals. The mean base line weight of all of the guinea pigs was 441 g (range, 394 to 549 g). One month later, the mean weight of those animals that had received a 4-week course of rifampin was 465 g (range, 422 to 590 g) as compared with 459 g (range, 404 to 562 g) for the controls not receiving the drug. There was no statistically significant difference among the groups immunized with BCG, DNCB, or SRBC.

Immunization with BCG. The effect of rifampin on cutaneous reactivity to PPD after sensitization to BCG is illustrated in Fig. 1. Skin reactivity appeared in both the control and experimental groups within 2 weeks and achieved a maximal response by 6 weeks. At this time, central necrosis frequently was evi-

FIG. 1. Effect of rifampin on cutaneous hypersensitivity to BCG. Symbols: ●, control animals; ○, rifampin-treated animals. Each point represents the mean and standard deviation of measurements of five animals per group.

dent. This reactivity persisted in the untreated group, but the response in the rifampin-treated animals decreased rapidly to 5 to 6 mm and remained hyporeactive until the drug was stopped 8 weeks later. Subsequently, skin reactions returned toward control levels within 2 weeks.

When rifampin treatment was initiated either 7 days before or 7 days after immunization, PPD skin reactions above 5 mm did not appear as long as the drug was continued.

Immunization with DNCB. The effects of rifampin on development of sensitivity to DNCB are summarized in Fig. 2 and Table 1. Suppression of established skin reactivity to this chemical agent was remarkably similar to that noted in guinea pigs sensitized to BCG (Fig. 1). In an attempt to explore further the temporal relationship between onset of treatment and immunization, groups of animals were begun on rifampin 7 days before and 2, 5, and 7 days after immunization (Table 1). None of the animals developed a significant reaction during treatment, and only those whose treatment was delayed until 7 days after primary DNCB sensitization reacted within 1 week after termination of the drug.

Immunization with SRBC. In experiments designed to test the effect of rifampin on immune induction, primary response, and secondary response to SRBC (Fig. 3), booster injections were administered approximately 6

TABLE 1. *Effect of initiating rifampin before and after immunization on cutaneous hypersensitivity to DNCB^a*

Day of onset of treatment ^b	Weeks after immunization	Skin thickness (mean $\pm$ SD in mm)
-7	0	1.4 $\pm$ 0.1
	1	1.4 $\pm$ 0.1
	2	1.6 $\pm$ 0.1
	3	1.4 $\pm$ 0.2
	4	1.4 $\pm$ 0.4
	4.5	1.6 $\pm$ 0.1
+2	5	1.5 $\pm$ 0.2
	0	1.5 $\pm$ 2.0
	1	1.5 $\pm$ 0.0
	2	1.6 $\pm$ 0.1
	3	1.8 $\pm$ 0.3
	4	1.7 $\pm$ 0.2
+5	4.5	1.9 $\pm$ 0.3
	5	1.8 $\pm$ 0.3
	0	1.6 $\pm$ 0.4
	1	1.6 $\pm$ 0.1
	2	2.0 $\pm$ 0.0
	3	1.9 $\pm$ 0.1
+7	4	2.0 $\pm$ 0.0
	4.5	2.0 $\pm$ 0.1
	5	2.2 $\pm$ 0.3
	0	1.5 $\pm$ 0.5
	1	1.7 $\pm$ 0.3
	2	1.6 $\pm$ 0.3
	3	1.6 $\pm$ 0.1
	4	1.8 $\pm$ 0.3
	4.5	3.1 $\pm$ 0.3
	5	4.5 $\pm$ 0.6

FIG. 2. *Effect of rifampin on cutaneous hypersensitivity to DNCB.* Symbols: ●, control animals; ○, rifampin-treated animals. Each point represents the mean and standard deviation of measurements of five animals per group.

^a 2,4-Dinitrochlorobenzene.

^b Rifampin was discontinued 4 weeks after immunization.

weeks after primary immunization or 7 days after cessation of the drug. The relationship between the onset of treatment and immunization was investigated by initiating 4-week courses of rifampin treatment according to the following plan: no treatment, 7 days prior to booster; 2, 3, and 4 days after primary immunization; and 7 days prior to primary immunization. Multiple replicate tests determined that the natural guinea pig anti-SRBC titer was 21 ± 7 CA₅₀ units.

When 10 control animals were immunized with SRBC (Fig. 4), five developed a clear response within 9 days (high responders), but no significant rise in titer was detected in the other five (low responders). An early strong secondary response appeared in all 10 animals, indicating that primary sensitization had occurred with or without a measurable rise in hemagglutinin antibody.

FIG. 3. Protocol of experiments to test the effect of a 4-week course of rifampin on immune induction, primary response, and secondary response to sheep red blood cells (SRBC).

FIG. 4. Primary and secondary response to sheep red blood cells (SRBC) in normal untreated animals. Note: the primary response has been divided into two groups. Symbols: $\circ$, high responders; $\bullet$, low responders (composed of five animals each). The secondary response of these same animals is demonstrated to the right. The natural anti-SRBC titer is depicted, $\blacktriangle$.

Although rifampin had no effect on the secondary response, it completely suppressed the primary reaction to SRBC irrespective of whether treatment was initiated 7 days before (nine animals) or 2 (four animals), 3 (five animals), or 4 (five animals) days after immunization. To test whether sensitization had occurred in the absence of a detectable response, we discontinued rifampin after 4 weeks and gave all 23 animals a booster injection.

Three patterns of hemagglutinin response followed. Within each group, some animals reacted to the booster with an early and high antibody response, some showed more delayed and intermediate titers, and some again failed to respond to a significant degree. Pooling the groups into these distinct categories (Fig. 5) showed that rifampin prevented sensitization to the initial antigen injections in 13 of 23 animals, as evidenced by the fact that they failed to develop a secondary-type response to the booster. The reaction of these 13 was virtually identical to the primary response in untreated control animals (Fig. 4); i.e., six developed delayed intermediate antibody levels and seven showed no significant change in titer.

To characterize these patterns further (Fig. 5), we incubated samples of positive serum with 2-mercaptoethanol and retitered them in comparison with the untreated sample. The derived IgM to IgG antibody ratio of the sera obtained from the more slowly responding animals was 14.2, indicating that the majority

FIG. 5. Hemagglutinin response to a second injection of sheep red cells (SRBC) in animals that had received rifampin during primary immunization (see text for protocols). Note the three patterns of response: a secondary type with high early rise in hemagglutinin titer, $\circ$, 10 animals; an intermediate primary type, $\bullet$, 6 animals; and a low insignificant response, Δ , 6 animals. The natural anti-SRBC titer is depicted, $\blacktriangle$.

was IgM and was consistent with a typical primary response. By contrast, the ratio in animals with a high early rise was 1.2, more typical of a secondary response with a greater fraction of IgG antibody.

DISCUSSION

Taken together, the results obtained with three antigens indicate that rifampin has immunosuppressive effects in the guinea pig. Three principal observations support this statement. First, when the drug was started prior to or shortly after immunization, skin test reactivity to PPD and DNCB failed to occur as long as treatment was continued. Second, rifampin treatment before or during sensitization suppressed the primary antibody response to SRBC, and only 10 of 23 animals developed a secondary response to a subsequent booster. This was confirmed by the finding that only the sera from these animals contained a proportion of IgG antibody consistent with a secondary response (11). When rifampin was delayed until 7 days after immunization, suppression of cutaneous hypersensitivity still occurred, but reactivity to DNCB did become evident after drug withdrawal. Finally, fully manifest skin hypersensitivity to both PPD and DNCB was suppressed during the course of treatment, an observation that parallels our clinical observations in 14 patients receiving 600 mg of rifampin per day (Mukerjee and Kasik, submitted for publication).

It is important to state that the immunosuppressive effects did not appear to be related to overt toxicity, since the guinea pigs treated with 20 mg per kg per day exhibited no change in weight gain as compared with controls, and Paunescu (5) noted anorexia and diarrhea only in those animals receiving greater than 50 mg per kg per day. Whether the immunosuppressive property is primarily related to rifampin,

to metabolic products of this drug, or to properties of other rifamycins contaminating clinically used rifampin, many of which are more cytotoxic (10), are important but unknown considerations.

ACKNOWLEDGMENTS

This investigation was supported by the Iowa Thoracic Society and the Veterans Administration.

LITERATURE CITED

1. Dajani, B. M., M. S. Canady, J. S. Thompson, and J. E. Kasik. 1972. Rifampicin: an immunosuppressant? *Lancet* (Letter to the Editor) 2:1094.
2. Devine, L. F., S. L. Rhode III, W. E. Pierce, D. P. Johnson, C. R. Hagerman, and R. O. Peckinpaugh. 1971. Rifampin: effect of two-day treatment on the meningococcal carrier state and the relationship to the levels of drug in sera and saliva. *Amer. J. Med. Sci.* 261:79-83.
3. Newman, R., B. Doster, F. J. Murray, and S. Ferebee. 1971. Rifampin in initial treatment of pulmonary tuberculosis. *Amer. Rev. Resp. Dis.* 103:461-476.
4. Nilsson, B. S. 1971. Rifampicin, an immunosuppressant? *Lancet* 2:374.
5. Paunescu, E. 1970. *In vivo* and *in vitro* suppression of humoral and cellular immunologic response by rifampicin. *Nature* (London) 228:1188-1190.
6. Raleigh, J. W. 1972. Rifampin in treatment of advanced pulmonary tuberculosis. *Amer. Rev. Resp. Dis.* 105:397-409.
7. Rosachino, F. 1967. Experiences in pediatrics with a new rifamycin. *Aggiorn. Pediat.* 19:107-110.
8. Serrou, B., C. Solassol, L. Karcenty, H. Joyeux, and H. Pujol. 1972. Action immunodepressive de la rifampicine. *Nouv. Presse Med.* 1:1295.
9. Severson, C. D., and J. S. Thompson. 1966. Quantitative semi-micro hemagglutination. *J. Immunol.* 96:785-789.
10. Smith, R. G., J. Whang-Peng, R. C. Gallo, P. Levine, and R. C. Ting. 1972. Selective toxicity of rifamycin derivatives for leukaemic human leucocytes. *Nature N. Biol.* 236:166-171.
11. Uhr, J., and M. S. Finkelstein. 1963. Antibody formation. *J. Exp. Med.* 117:457-477.
12. Valente, G., and A. Lavara. 1969. Clinical trial of rifampicin in pediatric practice: therapeutic activity and safety. *Clinica Pediatrica* 51:48-55.