Studies on Antinephritic Effect of Mizoribine (p-INN, Bredinin*), a New Immunosuppressive Agent, and Azathioprine (2) Effect on Crescentic-Type Anti-GBM Nephritis in Rats

Kyoko OKAMOTO*,**, Mikio ITO* and Yoshio SUZUKI*
*Department of Pharmacology, Faculty of Pharmacy, Meijo University, Tenpaku-ku, Nagoya 468, Japan
**Research Laboratories, Toyo Jozo Co., Ltd., Tagata-gun, Shizuoka 410-23, Japan
Accepted August 8, 1983

Abstract—Crescentic-type nephritis was induced in rats by immunizing with rabbit γ-globulin following i.v. injection of rabbit anti-rat glomerular basement membrane (anti-GBM) serum. The antinephritic effect of mizoribine was compared to that of azathioprine by administration from the 2nd day after the injection of anti-GBM serum to the 23rd day (Experiment I) and from the 15th to the 38th day (Experiment II). The experiment I assessment on the 24th day revealed that mizoribine at a dose of 7.5 mg/kg/day p.o. remarkably reduced plasma cholesterol level, wet kidney weight and glomerular histopathological changes (i.e., crescent formation and fibrinoid deposition). In addition, mizoribine at this dose also tended to reduce urinary protein excretion and the adhesion of capillary walls to Bowman’s capsule. At a dose of 5 mg/kg/day p.o., mizoribine significantly reduced kidney weight and crescent formation. On the other hand, azathioprine at a dose of 20 mg/kg/day p.o. had a tendency to reduce these biochemical and histopathological parameters. In the experiment II assessment on the 39th day, the effects of both drugs were somewhat diminished compared to those in experiment I. Mizoribine strikingly inhibited the crescent formation with 5 and 7.5 mg/kg/day p.o. and inhibited the fibrinoid deposition with a dose of 7.5 mg/kg/day p.o. Azathioprine at a dose of 20 mg/kg/day p.o. was prone to reduce histopathological parameters. The above data indicate that mizoribine may be a useful new immunosuppressive agent for rapidly progressive glomerulonephritis, which is characterized by severe glomerular lesions with the extensive formation of crescents.

Most human glomerulonephritis is clearly induced through an immunological mechanism, and immunosuppressive therapy is currently important for treatment of this disease. Mizoribine (p-INN, Bredinin®) [4-carbamoyl-1-β-D-ribofuranosyl imidazolium-5-olate] is a new immunosuppressive agent isolated from the culture media of Eupenicillium brefeldianum (1). The present investigators previously reported the beneficial effect of this drug on nephrotic-type glomerulonephritis induced in rats by two i.v. injections of rabbit anti-rat glomerular basement membrane (anti-GBM) serum (2). In spite of massive proteinuria and hyperlipemia, this nephritic model revealed mild glomerular lesions. Following studies with the experimental model of the nephrotic-type glomerulonephritis, the authors also established a model of crescentic-type glomerulonephritis closely resembling rapidly progressive glomerulonephritis (RPGN) in man, which was characterized by severe glomerular lesions with extensive formation of crescents. by injecting rabbit γ-globulin (γ-G) into the foot pads of rats that had been injected i.v. with anti-GBM serum (3). In the present study, the antinephritic effect of mizoribine on the crescentic-type nephritis in rats was assessed in comparison with that of azathioprine.
Materials and Methods

Drugs: Mizoribine (Toyo Jozo Co., Ltd.) and azathioprine (Tanabe Seiyaku Co., Ltd.) were used. Mizoribine was dissolved in distilled water, while azathioprine was suspended in 1% gum arabic solution. Both drugs were administered orally in a volume of 1 ml/100 g of body weight.

Animals: Male Wistar rats weighing 180 to 240 g were used in the experiments.

Experiment I: Crescentic-type nephritis was induced by immunizing rats that had received a nephritogenic dose of anti-GBM serum with rabbit \( \gamma \)-G according to a slight modification of the method reported previously (4). Forty-eight rats weighing approx. 240 g were administered 0.45 ml/animal of anti-GBM serum into the tail vein. On the day after the injection of anti-GBM serum, 24 hr urine samples were collected, and the rats were then divided into 4 groups of 12 animals, so that the average protein content in the 24 hr urine of each group became to the same level. After grouping, these animals were injected with 5 mg of rabbit \( \gamma \)-G in 0.25 ml of Freund’s complete adjuvant (FCA) into the hind foot pads. Three groups daily received 5 mg/kg and 7.5 mg/kg of mizoribine and 20 mg/kg of azathioprine, respectively, from the day of the injection of rabbit \( \gamma \)-G (the 2nd day) to the 23rd day. The remaining group, given p.o. only distilled water, served as the control. In addition, a non-treated (normal) group consisting of 7 rats weighing approx. 240 g was used in the experiment. Twenty-four hr urine samples from the 23rd to the 24th day were collected. Immediately following urine collection, blood was taken from the abdominal aorta into a centrifuge tube with the ratio of 9 parts blood to 1 part 3.13% trisodium citrate. The blood was then centrifuged at 3,000 rpm for 10 min at 4°C to obtain plasma.

Determinations of biochemical parameters of urine and plasma: The protein content in urine was determined by the method of Kingsbury et al. (4) and expressed as mg/24 hr urine. Plasma urea nitrogen (UN) and cholesterol (CL) contents were determined in accordance with the method of Searcy and Cox (5) and Zurkowski (6), respectively. Both results were expressed as mg/dl of plasma.

Kidney weight and assessment of histopathological parameters of kidneys: Kidneys were weighed, and the result was expressed as mg of the mean wet weight of both kidneys. For light microscopic study, the weighed kidneys were dehydrated and fixed by immersing the tissues stepwise into low to high concentrations of alcohol. The tissues were then embedded in paraffin and sectioned 2- to 3-\( \mu \) thick. The sections were stained with hematoxylin and eosin and Masson trichrome (MT). The degree of glomerular lesions was assessed in fifty glomeruli per section under light microscopy. The results were expressed as a percentage of the numbers of crescent formation, fibrinoid deposition and adhesion of capillary walls to Bowman’s capsule.
Statistical analyses: The data represent the mean±S.E., and the results were statistically evaluated by the Kruskal-Wallis test and multiple comparison except for the normal group.

Results
1. Effect of mizoribine and azathioprine administered from the heterologous phase on crescentic-type anti-GBM nephritis in rats (Experiment I)

   Body weight: Changes in body weight during the treatment with mizoribine or azathioprine are shown in Fig. 1. The average body weight of all groups was about 240 g at the start of drug treatment. In the control group, there was a body weight gain of 47 g during the period from the 2nd to the 23rd day after injection of anti-GBM serum. However, in the group given 7.5 mg/kg/day p.o. of mizoribine, no gain in body weight was observed during the course of treatment. A significant difference was recognized in the average body weight between both groups from the 9th day onwards after initiation of treatment. No significant difference was seen in the average body weight between mizoribine, 5 mg/kg/day p.o. or azathioprine, 20 mg/kg/day p.o.-treated group and the control group.

   Biochemical parameters of urine and plasma and kidney weight: Table 1 gives the results of mizoribine and azathioprine treatment on biochemical parameters of urine and plasma and kidney weight. The effects of both drugs were evaluated on the 24th day after injection of anti-GBM serum. The urinary protein excretion of the control group was 606.2±77.3 mg/day. Mizoribine at 5 and 7.5 mg/kg/day×22 p.o. inhibited the protein excretion by 20.8 and 38.7%, respectively, but no significant difference was noted between the mizoribine-treated groups and the control group. The plasma CL level of the control group was 3.2 times as high as that of the normal group. Mizoribine at 7.5 mg/kg/day×22 p.o. significantly reduced the CL level by 48.0%. The plasma UN level of the control group was only 1.5 times as high as that of the normal group. Mizoribine at a dose of 5 mg/kg/day×22 p.o. tended to reduce the UN level. The average wet weight of both kidneys of the control group was 1.6 times heavier than that of the normal group. Mizoribine at 5 and 7.5 mg/kg/day p.o. showed a percent inhibition of 90.6 and 92.5%, respectively, on kidney weight. On the other hand, azathioprine, a reference drug, at 20 mg/kg/day×22 p.o. did not show any significant effects on these biochemical parameters. This drug showed only insignificant inhibitory action on the kidney weight.

Fig. 1. Changes in body weight during treatment with mizoribine or azathioprine from the heterologous phase in crescentic-type anti-GBM nephritis in rats. Each plot denotes the mean value with S.E. of 9 to 12 rats, except for 7 rats in the normal group. * and ** indicate a significant difference from the control at P<0.05 and 0.01, respectively.
### Table 1. Effects of mizoribine and azathioprine administered from the heterologous phase on some parameters of urine and plasma and kidney weight in crescentic-type anti-GBM nephritis in rats

| Groups            | N  | Urinary protein (mg/24 hr urine) | Plasma urea nitrogen (mg/dl) | Plasma cholesterol (mg/dl) | Wet kidney weight (g) |
|-------------------|----|----------------------------------|-----------------------------|---------------------------|----------------------|
| Normal            | 7  | 14.6±1.7                         | 24.9±1.3                    | 51.1±2.6                  | 0.90±0.03            |
| Control           | 10 | 606.2±77.3                       | 37.3±4.1                    | 166.0±14.6                | 1.43±0.10            |
| Mizoribine 5 mg/kg/day p.o. | 12 | 483.1±52.8 (20.8)                | 29.5±3.3 (62.9)            | 148.8±18.0               | 0.95±0.03** (90.6)   |
| Mizoribine 7.5 mg/kg/day p.o. | 9  | 377.0±55.9 (38.7)                | 35.5±3.5 (58.7)            | 110.9±11.6* (48.0)       | 0.94±0.05** (92.5)   |
| Azathioprine 20 mg/kg/day p.o. | 9  | 507.6±61.0                       | 34.5±4.3                   | 149.9±18.3               | 1.15±0.06 (52.8)     |

Test drugs were given during the period from the 2nd to the 23rd day after injection of anti-GBM serum. Effects were evaluated on the 24th day. N: Number of rats. Results are the mean±S.E. The number in parentheses indicates the percent inhibition which is derived from the following formula: $\frac{C-T}{C-N} \times 100$ (C: Control, T: Test drug, N: Normal). * and ** indicate a significant difference from the control at P<0.05 and 0.01, respectively.

**Fig. 2.** Effects of mizoribine and azathioprine administered from the heterologous phase on glomerular histopathological parameters in crescentic-type anti-GBM nephritis in rats. Each column indicates the mean±S.E. Results of the normal group are 0. * and ** indicate a significant difference from control at P<0.05 and 0.01, respectively. For other references, see legend to Table 1.

**Histopathological parameters in kidneys:** Effects of mizoribine and azathioprine on glomerular histopathological parameters are illustrated in Fig. 2. On the 24th day after the induction of nephritis, the kidneys were observed under light microscopy. Mizoribine at 5 and 7.5 mg/kg/day×22 p.o. resulted in a significant inhibition of over 80% on the crescent formation. This drug at a dose of 7.5 mg/kg/day×22 p.o. also inhibited significantly the fibrinoid deposition by 87.2%, and at both doses, it reduced the adhesion of capillary walls to Bowman's capsule by about 70%; but there was no significant difference from the control. On the other hand, azathioprine at 20 mg/kg/day×22 p.o. only tended to reduce these three histopathological parameters. Photo 1 gives representative
micrographs of glomeruli from control rat and rats treated with mizoribine at 5 and 7.5 mg/kg/day × 22 p.o.

2. Effects of mizoribine and azathioprine administered from the autologous phase on crescentic-type anti-GBM nephritis in rats (Experiment II)

Body weight: Changes in body weight during the treatment with mizoribine or azathioprine are indicated in Fig. 3. When rats were grouped on the 15th day after injection of anti-GBM serum, the average body weights of the normal group and nephritic groups were approx. 280 and 250 g, respectively. In the group receiving 7.5 mg/kg of mizoribine, the body weight gain was only about 15 g during the period from the 15th to the 38th day, against 93 g in the control group. A

![Graph showing body weight changes](image)

Fig. 3. Changes in body weight during treatment with mizoribine or azathioprine from the autologous phase in crescentic-type anti-GBM nephritis in rats. Each plot denotes the mean value with S.E. of 9 to 10 rats, except for 5 rats in the normal group. * and ** indicate a significant difference from the control at P<0.05 and 0.01, respectively.

| Test drugs | Body weight gain (g) |
|------------|----------------------|
| Control    | 93 ± 4.5             |
| Mizoribine 5 mg/kg/day p.o. | 15 ± 2.1  |
| Mizoribine 7.5 mg/kg/day p.o. | 10 ± 1.2   |
| Azathioprine 20 mg/kg/day p.o. | 0 ± 0.2    |

### Table 2. Effects of mizoribine and azathioprine administered from the autologous phase on some parameters of urine and plasma and kidney weight in crescentic-type anti-GBM nephritis in rats

| Groups          | N  | Urinary protein (mg/24 hr urine) | Plasma urea nitrogen (mg/dl) | Plasma cholesterol (mg/dl) | Kidney weight (g) | Wet kidney weight (%) |
|-----------------|----|---------------------------------|-----------------------------|---------------------------|-------------------|-----------------------|
| Normal          | 5  | 13.1 ± 1.7                      | 15.7 ± 1.0                  | 49.6 ± 3.9                | 0.99 ± 0.09       |                      |
| Control         | 10 | 228.2 ± 56.3                    | 23.7 ± 1.1                  | 88.1 ± 9.9                | 1.26 ± 0.09       |                      |
| Mizoribine 5 mg/kg/day p.o. | 10 | 145.3 ± 32.9                    | 25.0 ± 2.0                  | 62.1 ± 6.4                | 1.08 ± 0.05       |                      |
|                 |    | (38.5)                          | (67.4)                      | (66.7)                    | (66.7)            |                      |
| Mizoribine 7.5 mg/kg/day p.o. | 9  | 86.2 ± 33.2                     | 22.8 ± 1.8                  | 56.1 ± 4.5                | 0.98 ± 0.05       |                      |
|                 |    | (66.0)                          | (82.9)                      | (103.7)                   | (103.7)           |                      |
| Azathioprine 20 mg/kg/day p.o. | 10 | 163.9 ± 32.0                    | 29.0 ± 1.7                  | 81.6 ± 7.3                | 1.19 ± 0.07       |                      |
|                 |    | (29.9)                          | (25.9)                      | (25.9)                    |                   |                      |

Test drugs were given during the period from the 15th to the 38th day after injection of anti-GBM serum. Effects were evaluated on the 39th day. N: Number of rats. Results are the mean ± S.E. The number in parentheses indicates the percent inhibition which is derived from the following formula: C−T x 100 / C−N (C: Control, T: Test drug, N: Normal).
significant difference was found in the average body weight between the two groups after the 10th day of treatment. No significant difference was recognized in the average body weight between other treated groups and the control group.

Biochemical parameters of urine and plasma and kidney weight: Table 2 lists the effects of mizoribine and azathioprine on biochemical parameters of urine and plasma and kidney weight. Both drugs were given from the 15th day after the injection of anti-GBM serum, and the effects were evaluated on the 39th day. Mizoribine at 5 and 7.5 mg/kg/day×24 p.o. reduced urinary protein excretion by 38.5 and 66.0%, the plasma CL level by 67.4 and 82.9%, and kidney weight by 66.7 and 103.7%, respectively. However, no significant difference was seen in these parameters in the mizoribine-treated group and the control group. On the other hand, azathioprine at 20 mg/kg/day×24 p.o. was ineffective in inhibiting these parameters.

Histopathological parameters in kidneys: Effects of mizoribine and azathioprine on glomerular histopathological parameters are shown in Fig. 4. At the histopathological observation on the 39th day after the induction of nephritis, mizoribine at 5 and 7.5 mg/kg/day×24 p.o. indicated a significant inhibition of 72.9 and 82.6%, respectively, on the crescent formation. At a dose of 7.5 mg/kg/day×24 p.o., mizoribine caused a potent 95.2% inhibition of fibrinoid deposition. Mizoribine at both doses tended to reduce the adhesion of capillary walls to Bowman's capsule. Azathioprine at 20 mg/kg/day×24 p.o. revealed only insignificant inhibition on the crescent formation and fibrinoid deposition. Photo 2 gives representative micrographs of glomeruli from control and mizoribine-treated rats.

Discussion

The glomerular injury of anti-GBM nephritis induced in rats (7) or rabbits (8) by i.v. injection of heterologous antiserum against GBM antigen is well-known to consist of two phases. The heterologous phase (primary or passive phase) is due to immediate fixation of the injected anti-GBM antibody to the glomeruli. On the other hand, the autologous phase (secondary or active phase) is due to a reaction between host antibody against the injected anti-GBM serum protein and the anti-GBM antibody already fixed to the glomeruli. In the present study, the crescentic-type nephritis in rats employed for assessing the drug effect was induced by immunizing with rabbit r-G following the
Antinephritic Effect of Mizoribine

Injection of anti-GBM serum (rabbit anti-serum against rat GBM). As reported earlier (3), immunization with rabbit γ-G in this model may result in severe glomerular injury with extensive formation of crescents by enhancing the immune response in the

Photo 1. Kidney sections from control rat (a), mizoribine 5 mg/kg/day p o. (b) - and 7.5 mg/kg/day p o. (c)-treated rats on the 24th day after injection of anti-GBM serum MT stain (×200)

Photo 2. Kidney sections from control rat (a), mizoribine 5 mg/kg/day p o. (b) - and 7.5 mg/kg/day p o. (c)-treated rats on the 39th day after injection of anti-GBM serum MT stain (×200)
autologous phase via the persistent formation of host antibody against rabbit r-G.

In experiment I, the antinephritic effect of mizoribine was compared to that of azathioprine by starting the administration almost simultaneously with the onset of nephritis, that is, from the heterologous phase. The doses of both drugs were the same as those used in the nephrotic-type nephritis of rats reported previously (2). At the assessment on the 24th day after injection of anti-GBM serum, mizoribine at a dose of 7.5 mg/kg/day × 22 p.o. remarkably reduced the plasma CL level, wet kidney weight and glomerular histopathological changes such as crescent formation and fibrinoid deposition. At the same dose, it also tended to reduce the urinary protein excretion and the adhesion of capillary walls to Bowman's capsule. At a dose of 5 mg/kg/day × 22 p.o., mizoribine also significantly reduced kidney weight and crescent formation. On the other hand, azathioprine at a dose of 20 mg/kg/day × 22 p.o. only tended to reduce these biochemical and histopathological parameters. In experiment II, test drugs were started from the 15th day after induction of nephritis, that is, from the autologous phase. At the assessment on the 39th day, the effects of both drugs were somewhat less than those in experiment I. Mizoribine showed apparent inhibitory effects on the crescent formation and fibrinoid deposition with a dose of 7.5 mg/kg/day × 24 p.o. This drug at both doses tended to reduce other parameters. Azathioprine at 20 mg/kg/day × 24 p.o. only tended to reduce histopathological parameters. Thus, mizoribine showed a more marked effect than azathioprine in both experiments. There are several reports dealing with the effects of immunosuppressive agents on experimental glomerulonephritis. Immunosuppressive agents have been shown to have little or no effect on autologous immune complex (AIC) glomerulonephritis of rats only when administered before or simultaneously with the onset of nephritis. From the above reports, one can deduce that immunosuppressive agents no longer exert a beneficial effect on nephritis when the treatment is begun at a later stage of the disease. However, in the present experiments, mizoribine showed a desirable effect on crescentic-type nephritis when the treatment was begun not only simultaneously with the induction of the disease but also about two weeks after the induction, at a later stage. The present authors observed that the serum antibody titer against rabbit r-G in this model rapidly elevated from the 15th day after the induction of nephritis, and thereafter remained elevated up to the 40th day (K. Okamoto et al., unpublished data). Therefore, it is reasonable to presume that the antinephritic action of mizoribine treatment beginning at a later stage could prevent the host antibody formation against rabbit r-G. Mizoribine is thought to inhibit humoral immune response through the suppression of the generation of helper T cells, memory helper T cells and memory B cells when administered at the inductive phase (13). Our results suggest that mizoribine may be valuable for the treatment of nephritis in the active stage of immune response or RPGN with crescents in clinical application. However, it is noteworthy that no body weight gain was observed during the treatment with 7.5 mg/kg/day p.o. of this drug. The antinephritic effect of mizoribine could not be considered to be due to the decrease of the gain in body weight because there was no correlation with body weight and the antinephritic effect in rats treated with mizoribine. Accordingly, a safe and effective treatment with mizoribine resulted in a beneficial effect on AIC glomerulonephritis in rats when it was started simultaneously with the immunization procedure or when immune deposits appeared along the GBM. However, no beneficial effect of the triple-drug treatment could be demonstrated when the treatment was started from the later stage of the disease when proteinuria was fully developed.

According to Feenstra et al. (12), azathioprine was effective in preventing heterologous immune complex (HIC) glomerulonephritis of rats only when administered before or simultaneously with the onset of nephritis. From the above reports, one can deduce that immunosuppressive agents no longer exert a beneficial effect on nephritis when the treatment is begun at a later stage of the disease. However, in the present experiments, mizoribine showed a desirable effect on crescentic-type nephritis when the treatment was begun not only simultaneously with the induction of the disease but also about two weeks after the induction, at a later stage. The present authors observed that the serum antibody titer against rabbit r-G in this model rapidly elevated from the 15th day after the induction of nephritis, and thereafter remained elevated up to the 40th day (K. Okamoto et al., unpublished data). Therefore, it is reasonable to presume that the antinephritic action of mizoribine treatment beginning at a later stage could prevent the host antibody formation against rabbit r-G. Mizoribine is thought to inhibit humoral immune response through the suppression of the generation of helper T cells, memory helper T cells and memory B cells when administered at the inductive phase (13). Our results suggest that mizoribine may be valuable for the treatment of nephritis in the active stage of immune response or RPGN with crescents in clinical application. However, it is noteworthy that no body weight gain was observed during the treatment with 7.5 mg/kg/day p.o. of this drug. The antinephritic effect of mizoribine could not be considered to be due to the decrease of the gain in body weight because there was no correlation with body weight and the antinephritic effect in rats treated with mizoribine. Accordingly, a safe and effective treatment with mizoribine
is now under investigation by intermittently administering this drug to rats with crescentic nephritis in our laboratory.

References

1. Mizuno, K., Tsujino, M., Takada, M., Hayashi, M., Atsumi, K., Asano, K., and Matsuda, T.: Studies on bredinin. I. Isolation, characterization and biological properties. J. Antibiot. (Tokyo) 27, 775–782 (1974)

2. Okamoto, K., Ito, M., and Suzuki, Y.: Studies on antinephritic effect of mizoribine (p-INN, Bredinin®), a new immunosuppressive agent, and azathioprine (1). Effect on the nephrotic type of anti-GBM nephritis in rats. Japan. J. Pharmacol. 33, 541–548 (1983)

3. Ito, M., Yamada, H., Okamoto, K., and Suzuki, Y.: Crescentic type nephritis induced by anti-glomerular basement membrane (GBM) serum in rats. Japan. J. Pharmacol. 33, 1145–1154 (1983)

4. Kingsbury, F.B., Clark, C.P., Williams, G., and Post, A.L.: The rapid determination of albumin in urine. J. Lab. Clin. Med. 11, 981–989 (1926)

5. Searcy, R.L., and Cox, F.M.: A modified technique for ultramicro estimations of urea nitrogen. Clin. Chim. Acta 8, 810–812 (1963)

6. Zürkowski, P.: A rapid method for cholesterol determination with a single reagent. Clin. Chem. 10, 451–463 (1964)

7. Ito, M., Nagamatsu, T., and Suzuki, Y.: Pharmacological studies on experimental nephritic rats (10). Changes in coagulation-fibrinolysis system in the course of anti-GBM induced nephritis. Japan. J. Nephrology 23, 297–308 (1981) (Abs. in English)

8. Border, W.A., Wilson, C.B., and Dixon, F.J.: Failure of heparin to affect two types of experimental glomerulonephritis in rabbits. Kidney Int. 8, 140–148 (1975)

9. Lim, V.S. and Spargo, B.H.: Immunosuppressive treatment of autologous immune complex nephritis in rats. J. Lab. Clin. Med. 81, 661–670 (1973)

10. Kupor, L.R., Lowance, D.C., and McPhaul, J.J.: Single and multiple drug therapy in autologous immune complex nephritis in rats. J. Lab. Clin. Med. 87, 27–36 (1976)

11. Fleuren, G.J., and Hoedemaeker, P.H.: Triple-drug treatment of autologous immune complex glomerulonephritis. Clin. Exp. Immunol. 41, 189–195 (1980)

12. Feenstra, K., van der Lee, R., Greben, H.A., Arende, A., and Hoedemaeker, P.H.: Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigen II. Influence of medication with prednisolone and azathioprine: A histologic and immunohistologic study at the light microscopic and the ultrastructural level. Lab. Invest. 32, 243–250 (1975)

13. Yoshizawa, M., Tsujino, M., Mizuno, K., and Hayano, K.: Immunosuppressive effect of mizoribine II. Suppression of humoral cellular immune responses. Clin. Immunol. 14, 561–570 (1982) (Abs. in English)