Preliminary Results of Short-Term Combination Immunosuppressions of Mizoribine, Azathioprine, and Prednisolone with Pretreatment to Canine Kidney Transplantation

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ABSTRACT. Serial combinations of immunosuppressive drugs (mizoribine: Mi, azathioprine: Az, and prednisolone: Pr) were administered for renal heterotopic allotransplantation in 50 mixed-breed dogs and 4 beagle dogs. The dogs were randomly divided in 5 groups depending upon immunosuppressive protocols. All immunosuppressive protocols were started at 5 days prior to transplantation and discontinued on the eleventh day after transplantation. The mean survival time for the allograft recipients in the group receiving Az and Pr [Az (2,5) & Pr group; Az dosage, 2.5 mg/kg once a day with Pr] was 16.2 ± 2.4 days and in the group receiving Az, Mi, and Pr (Mi & Az & Pr group; Mi dosage, 5.0 mg/kg once a day with Az and Pr), it was 14.0 ± 2.6 days. These results were significantly longer than the control group (mean survival time, 8.7 ± 2.4 days; P<0.01). There was no statistical difference in survival time between these two groups. Groups with combinations of Az and Pr had significant elevations of hepatic enzymes (ALT and ALP) during the 7 days of immunosuppressive treatment after kidney transplantation. The Mi & Az & Pr group had lower elevations of hepatic enzymes than groups with combinations of Az and Pr. The combination immunosuppression of Mi, Az, and Pr with pre-treatment for canine kidney transplantation revealed relatively fewer side-effects for the liver and longer survival-time.—KEY WORDS: canine, combination immunosuppression, kidney transplantation.


Chronic end-stage renal diseases are common in dogs. Treatments for end-stage renal failure, such as hemodyalisis and continuous ambulatory peritoneal dialysis, have been reported in veterinary medicine [14]. However, there are quiet few effective procedure for maintaining long-term renal function [14]. The high success rate of kidney transplantation in humans gives patients having end-stage renal diseases a good quality of life [13].

There have been many investigations and clinical trials of kidney transplantation for the treatment of renal failure in dogs [1, 6-8]. There are quite few effective methods for resolving complications from rejection of transplanted kidneys at this point [5]. In this study, mizoribine (4-carbamoyl-1-β-D-ribofuranosilamidazolium-5-olate), azathioprine, and prednisolone were the immunosuppressive drugs used. Mizoribine is a nucleoside antibiotic from the culture filtrate of Eugenicillium brefeldianum [10]. The immunosuppressive mechanism is the inhibition of ribonucleic acid and deoxyribonucleic acid synthesis causing lymphocyte toxicities [11]. Mizoribine also has less hepatic toxicity than azathioprine [10].

Cyclosporine has been widely used as an immunosuppressive drug in human organ transplantations [3]. However, renal toxicity has been reported in long-term survival patients and it is the most expensive immunosuppressive drug for canine renal transplantation when used in effective immunosuppressive dosages [12]. Mizoribine is a relatively inexpensive drug and its toxicity is lessened when used with combinations of other immunosuppressive drugs. Therefore, it is a better choice.

The purpose of this short-term study is to determine the efficacy and selection of combination immunosuppressive protocols with pretreatments for canine kidney transplantation to increase survival times and decrease side-effects, especially those affecting the liver and perform the long-term combination immunosuppression study.

MATERIALS AND METHODS

Beagle and mixed-breed dogs were obtained. The dogs were randomly divided into 5 groups and each received a different immunosuppressive protocol. The mean body weight was 12.1 ± 4.1 kg, ranging from 5.8 to 24 kg. The body weight of recipients (R) and donors (D) were paired as equally as possible in each group. R/D ratios (mean ± SD) are listed in Table 1. There was no significant statistical difference in body weight between each group. The dogs were also paired on the basis of negative results of major and minor cross-matches which were examined that recipient plasma versus donor red blood cells and donor plasma versus recipient red blood cells were evaluated for the presence of hemolysis and macroscopic and microscopic agglutination. Commercial dry food and water were provided.

Anesthesia was induced intravenously with thiometal sodium (25 mg/kg) and maintained with halothane in combination with oxygen. Recipient and donor dogs were treated with oral antibiotics (amoxicilin 20 mg/kg BID) 5 days prior to the operation, intravenous ampicilin (25 mg/kg TID) for 3 days postoperatively, and then switched
Table 1. Materials of short-term canine kidney transplantation

<table>
<thead>
<tr>
<th>Group</th>
<th>B.W. (Mean±SD)</th>
<th>R/D ratio (Mean±SD)</th>
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<tbody>
<tr>
<td>1</td>
<td>Recipient (n:6)</td>
<td>12.9±2.0</td>
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<tr>
<td></td>
<td>Donor (n:6)</td>
<td>14.1±4.7</td>
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<tr>
<td>2</td>
<td>Recipient (n:5)</td>
<td>12.7±4.2</td>
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<td></td>
<td>Donor (n:5)</td>
<td>13.3±6.3</td>
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<td>3</td>
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<td></td>
<td>Donor (n:6)</td>
<td>9.7±4.0</td>
</tr>
<tr>
<td>4</td>
<td>Recipient (n:5)</td>
<td>14.9±4.5</td>
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<td></td>
<td>Donor (n:5)</td>
<td>15.2±4.8</td>
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<tr>
<td>5</td>
<td>Recipient (n:5)</td>
<td>11.2±2.4</td>
</tr>
<tr>
<td></td>
<td>Donor (n:5)</td>
<td>10.4±2.2</td>
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to the oral protocol used before surgery at the same dosage until the end of study. Lactated Ringer’s solution (2 ml/kg/hr) was transfused intravenously during surgery and 1 mg/kg furosemide was injected intravenously after the re-perfusion of renal vessels. The donors’ kidney was flushed with 50 ml of 5% glucose lactated Ringer’s solution (room temperature) and another 50 ml of the same solution cooled to 4°C. The removed kidney was kept in ice cold, 5% glucose lactated Ringer’s solution until renal vascular anastomosis. The donor’s kidney was placed in the recipients’ lower abdominal cavity. End-to-end, renal-to-external iliac artery and end-to-side, renal-to-common iliac vein anastomoses were performed. After anastomosis, the ureter and urinary bladder were anastomosed with Murry’s method. The recipients’ own renal artery, vein, and ureter were ligated. For 3 days after transplant surgery, recipients were not given any food or water, but were given 60 ml/kg/day of 5% glucose lactated Ringer’s solution intravenously. On the fourth day after surgery, feeding was started gradually.

Five groups of transplantations, including the control group, were performed depending upon immunosuppressive drugs and dosages (Fig. 1). All immunosuppressive protocols were started at 5 days prior to transplantation in both recipients and donors and discontinued on the eighth day after transplantation.

Az(2.5) & Pr group: Daily oral administration of azathioprine\textsuperscript{a} (dosage, 2.5 mg/kg of body weight once a day) was started at 5 days prior to transplantation. Prednisolone (dosage, 1.0 mg/kg of body weight once a day) was started 2 days prior to transplantation. On the day of transplantation, azathioprine was reduced to 1.0 mg/kg once a day. Both drugs were given until the seventh day after transplantation.

Az(5.0) & Pr group: Daily azathioprine (dosage, 5 mg/kg once a day) was administered prior to surgery and reduced to 1.0 mg/kg once a day after surgery. Prednisolone was administered according to the method used for group receiving Az and Pr.

Mi(5.0) & Pr group: Mizoribine\textsuperscript{b} (dosage, 5mg/kg of body weight once a day) was administered 5 days prior to surgery and reduced to 2.5 mg/kg on the day of transplantation.

Mi & Az & Pr group: Mizoribine and prednisolone were administered according to the same method used for the Mi(5.0) & Pr group. Azathioprine (dosage, 2.0 mg/kg once a day) was started 2 days prior to surgery and reduced to 1.0 mg/kg on the day of transplantation. These immunosuppressive drugs were administrated daily at least 60 minutes prior to feeding.

The clinical status of the dogs were observed daily and drug administration and blood sampling were performed prior to feeding. Blood was obtained for analysis every day. Complete blood counts, serum creatinine concentra-

\textsuperscript{a} (Imuran, Burroughs Wellcome Co.)

\textsuperscript{b} (Bredenine, Toyo Jozo Co. Ltd., Tokyo, Japan)
tion, BUN, serum alanine aminotransferase activity (ALT), and alkaline phosphatase activity (ALP) were analyzed until end of the study.

The dogs that became severely azotemic were euthanatized.

Statistical analysis was performed using Student's paired and unpaired t-tests, as appropriate. For all statistical analyses, a P value of 0.05 or less was considered significant.

RESULTS

The mean survival times of each group were analyzed for statistical differences using Student's unpaired t-test (Table 2; Fig. 2). Both the Az(2.5) & Pr group (mean survival time, 16.2±2.4 days) and the Mi & Az & Pr group (mean survival time, 14.0±2.6 days) had significantly longer survival times compared to the control group (mean survival time, 8.7±2.4 days; P<0.01). There was no significant differences in survival time between these groups.

There were no significant change in clinical signs during pretreatment in all dogs. Feeding began on the fourth day after transplantation. At that time, there was no significant change of appetite in any dog. As renal function decreased, anorexia, vomiting and diarrhea became worse. Severity and duration of these clinical signs depended upon the degree and duration of renal dysfunction.

There was no statistical difference of ischemic times during transplantation surgery between groups.

There were mild decreased packed cell volumes and red blood cell counts in all groups because of daily blood sampling. There were mild increased white blood cell counts for 1 to 3 days after transplantation in all groups. There were no severe decreased white blood cell counts (less than 4,000/μl) during survival periods in any dogs.

The mean creatinine level of the control group gradually increased after transplantation (Fig. 3). There were no significant changes in the Az(2.0) & Pr and Mi & Az & Pr groups during the 7 days of immunosuppressive treatments (Fig. 3). In the Az(5.0) & Pr group, there were fewer increased mean creatinine levels at time of death than in other groups even though the group's mean survival time was the shortest. After discontinuation of immunosuppressive treatment, creatinine levels gradually increased in the Az(2.0) & Pr and Mi & Az & Pr groups. Changes in BUN levels in each group were mostly the same as changes in creatinine levels (Fig. 4).

![Fig. 2. Survival time in short-term immunosuppression. Remarks; *: vs Control (P<0.01) N.S.: Not significant](image)

![Fig. 3. Changes of serum creatinine in kidney transplantation with short term immunosuppression. Small numbers over the SD bars represent changes in the number of dogs. * and ** marks indicate statistical significances comparing to data of 5 days prior to surgery.](image)

There were significantly increased mean activities of alkaline phosphatase (ALP) in the control, Az(2.5) & Pr, and Az(5.0) & Pr groups after transplantation compared to their levels at 5 days prior to kidney transplantation.

<table>
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<th>Table 2. Survival time of short-term canine kidney transplantation</th>
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Fig. 4. Changes of BUN in canine kidney transplantation with short term immunosuppression. Small numbers over the SD bars represent changes in the number of dogs. * and ** marks indicate statistical significances comparing to data of 5 days prior to surgery.

Fig. 6. Changes of mean alanine aminotransferase activity in canine kidney transplantation with short term immunosuppression. Small numbers over the SD bars represent changes in the number of dogs. * and ** marks indicate statistical significances comparing to data of 5 days prior to surgery.

(Fig. 5). The Az (2.0) & Pr and Az(5.0) & Pr groups had significantly increased mean ALP activities at the day of or 1 day after transplantation and remained at significantly high levels in most cases until the end of the study. There was also significantly increased mean activity of alanine transaminase (ALT) in the control, Az(2.0) & Pr and Az(5.0) & Pr groups (Fig. 6). The highest activity of ALT was in the Az(5.0) & Pr group at the day of transplantation. There were fewer changes in ALP and ALT levels in groups receiving mizoribine.

DISCUSSION

There are many experimental and clinical reports of canine kidney transplantation using cyclosporine, azathioprine, and prednisolone [1, 4, 5]. However, each immunosuppressive drug has side-effects [3, 16]. Cyclosporine can cause renal toxicity when used for long-term immunosuppression and eventually other drugs must be substituted or the dosage must be reduced and used in combination with other immunosuppressive drugs [3].

Fig. 5. Changes of mean alkaline phosphatase activity in canine kidney transplantation with short term immunosuppression. Small numbers over the SD bars represent changes in the number of dogs. * and ** marks indicate statistical significances comparing to data of 5 days prior to surgery.
Azathioprine can cause hepatic toxicity, so it can not be used at high doses or for long-term immunosuppression [2]. Therefore, combinations of azathioprine, prednisolone, and mizoribine have been investigated to study the effects of reduced doses on survival time and the ability of these combinations to immunosuppress different portions of rejection mechanisms [15, 16].

Mizoribine is relatively inexpensive. Therefore, it is acceptable immunosuppressive drug for long-term usage in clinical veterinary field and has fewer hepatic side-effects than azathioprine [8, 9]. We established serial combination immunosuppressive protocols with mizoribine, azathioprine, and prednisolone and accompanied by pretreatment for recipients and donors. This study is a trial to establish the most effective immunosuppressive protocol for canine kidney transplantation for the long-term. We performed pretreatment in recipients and donors for 5 days before transplantation to further prolongation of survival time.

The Az(5.0) & Pr group had the shortest mean survival time, which was same as the control group. Three dogs out of 6 at the day of death had serum creatinine levels less than 2 mg/dl and BUN levels less than 50 mg /dl in the Az(5.0) & Pr group. However, there were significantly high ALT and ALP activities in the same group. ALT activity was highest at the day of transplantation and gradually decreased.

Azathioprine was tested for toxicity (dosage, 5 mg/kg/day) for 62 days and no significant changes in ALT and ALP activities occurred before the forty-ninth day [9]. However, there were significantly increased ALT and ALP levels in the control group in this study. It might be due to differences in the surgical procedure; dissection of renal vessels and anastomosis were performed close to adrenal glands. Therefore, it is quite possible that the adrenal glands were stimulated. Significant elevations of ALT and ALP in the Az(5.0) & Pr group appeared to be caused by the hepatic side-effects of azathioprine and prednisolone even though the rejection reactions of transplanted kidneys were suppressed.

The Mi(5.0) & Pr group had the second shortest survival time which was no different from that of the control group. Mean activities of ALT and ALP gradually increased during the 7 days of immunosuppression in this group. The protocol and dosage of mizoribine appeared to have inadequate immunosuppressive effects and was not effective for renal transplantation. Mizoribine itself might not be sufficiently immunosuppressive, as has been reported [16]. However, this group had the least changes in ALT and ALP. A study of the toxicity of mizoribine (dosage, 5 mg/kg/day) revealed no significant changes in ALT activity [9]. According to the same study, mizoribine itself appeared to have fewer hepatic side-effects than azathioprine [9].

The Az(2.5) & Pr and Mi & Az & Pr groups had significantly longer mean survival times than the control group. However, there was no difference in mean survival time between these groups. There were minimal changes in creatinine (less than 2 mg/dl) and BUN levels (less than 50 mg/dl) in the Az(2.5) & Pr and Mi & Az & Pr groups during the 7 days of immunosuppressive treatment. After discontinuing immunosuppression, both levels increased by the fourth day in the Az(2.5) & Pr group and by the second day in the Mi & Az & Pr group. One study revealed a mean survival time of 43.9±5.1 days with azathioprine (dosage, 5 mg/kg/day) and 53.9±13.9 with mizoribine, azathioprine, and prednisolone for kidney transplantations in beagle dogs [16]. Our results cannot be compared with the results of that study because 5 days of pretreatment and only 7 days of immunosuppression following transplantation were performed in our study. ALT activities were increased in both groups and there was a decreased tendency towards ALT activity in the Mi & Az & Pr group during the immunosuppressive period. ALT levels decreased by the eleventh day in the Az(2.5) & Pr group. The Az(2.5) & Pr group had ALP levels that were two times higher than the Mi & Az & Pr group. ALP activity remained at more than 200 IU/l in all dogs during the entire study. There were significant differences in ALP activity levels between before and after the administration of prednisolone. This might be explained by prednisolone’s ability to produce ALP in bile duct epithelial cells.

Hepatic toxicities of azathioprine have been reported [9]. Our study revealed that azathioprine and prednisolone were relatively adequate when used for short-term immunosuppression, but it appears that this protocol may cause more significant liver damage when it is used for long-term immunosuppression. Immunosuppression using a combination of mizoribine, azathioprine, and prednisolone has also been reported [15]. Results were better with regards to the prolongation of allograft function, degrees of hepatic damage, bone marrow suppression, and infections in human beings [15].

This short-term study revealed that a combination of mizoribine, azathioprine, and prednisolone with pretreatment of recipients and donors for immunosuppression (the Mi & Az & Pr group) had fewer hepatic side-effects and was a relatively effective immunosuppressant treatment method for canine kidney transplantation even though the period of immunosuppression was only 12 days. The long-term study is needed to prove that survival is prolonged in dogs receiving kidney transplantations with this immunosuppressive protocol.

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REFERENCES


