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THE EFFECT OF A NEW IMMUNOSUPPRESSIVE DRUG, BREQUINAR SODIUM, ON HEART, LIVER, AND KIDNEY ALLOGRAFT REJECTION IN THE RAT[1]

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Brequinar sodium (BQR) prevents cell proliferation by virtue of its inhibition of de novo pyrimidine biosynthesis. BQR is capable of inhibiting immune responses in vitro and is effective in suppressing the development of contact sensitivity and adjuvant arthritis in rodent models. Based on the antiproliferative and immunosuppressive capacity of BQR, we have evaluated the efficacy of BQR in preventing allograft rejection utilizing experimental models of heterotopic heart and kidney and orthotopic liver transplantation in an MHC and non-MHC mismatched ACI→LEW rat strain combination.

The immunosuppressive activity of BQR is illustrated by its ability to inhibit the development of delayed-type hypersensitivity to DNFB in mice. When BQR was administered orally throughout the sensitization and elicitation phases of the DNFB contact sensitivity response, it was found to be a potent immunosuppressant with an ED_{50} value of 0.5 mg/kg. This immunosuppressive activity is also seen in vitro, where BQR is capable of inhibiting the mixed lymphocyte response between allogeneic ACI and LEW rat strains with an IC_{50} of 150 ng/ml.

The immunosuppressive activity of BQR is highly effective in prolonging heart, liver, and kidney allograft survival in the rat. Cardiac allografts are not rejected during the period of drug treatment at dosage levels of 12 to 24 mg/kg orally three times weekly. The grafts survive until the drug is discontinued (30 days post-transplantation), and the grafts are then rejected approximately 14 days later. Liver and kidney allografts are permanently accepted by approximately 50 to 90% of the recipient rats following 30 days of treatment with BQR at 12 mg/kg. The tolerance that is induced to the liver grafts extends in the majority of animals to greater than 250 days and is specific for the donor ACI strain. Challenge of long-term liver graft survivors with donor cardiac grafts is associated with permanent survival of donor, but not third-party, heart grafts. Combination therapy consisting of suboptimal doses of BQR and CsA demonstrates that the combination of these two immunosuppressive drugs results in an increased efficacy in prolonging graft survival.

The results of these allograft experiments demonstrate that this new immunosuppressive agent is highly effective in preventing allograft rejection in the rat. The antiproliferative activity of BQR is effective for inhibiting T-lymphocyte–mediated immune responses, and Brequinar sodium should be an important addition to a polytherapeutic approach in the treatment of organ graft rejection.

The introduction of effective immunosuppressive agents has made the transplantation of solid organs a viable and important therapeutic alternative for end-stage disease of these organs. The most useful of these immunosuppressive agents, cyclosporine A, is one of a family of fungal metabolites that has proven to be a potent and novel immunosuppressive agent in both animal models and clinical transplantation (*1–4*). While CsA has had an important impact on the survival of transplanted organs, adverse side effects have been recognized, and they represent a serious limitation to the long-term use of this immunosuppressive drug (*5, 6*).

The pharmacologic agent Brequinar sodium (BQR) is an anticancer drug that inhibits cell proliferation by virtue of its inhibition of the activity of dihydroorotate dehydrogenase and de novo pyrimidine biosynthesis (*7*). The application for the use of BQR in clinical medicine has been for the treatment of metastatic tumors and leukemias. Further experimentation with this compound, however, has demonstrated that it is capable of modulating immune responses and effective in suppressing the development of contact sensitivity and adjuvant arthritis in rodent models.[5] The drug acts most efficiently in the early stages of the development of the adjuvant arthritis and the induction of contact sensitization. When compared with other immunosuppressive drugs (i.e., CsA or azathioprine), BQR is more effective in preventing the development of these immunological reactions (*8*).

Based on the antiproliferative nature of these compounds and preliminary indications of the immunosuppressive capacity of BQR, our laboratory has evaluated the efficacy of BQR in preventing allograft rejection, utilizing surgical models of heart, liver, and kidney transplantation in an MHC and non-MHC mismatched rat strain combination (ACI→LEW). Our results have demonstrated that this novel immunosuppressive agent is highly effective in preventing allograft rejection in the rat. Our preliminary in vitro and in vivo data demonstrating increased efficacy of combinations of BQR and CsA suggest that BQR may represent an agent that will be an important addition to a

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polytherapeutic approach in the treatment of organ graft rejection.

MATERIALS AND METHODS

Animals. Rats: Adult LEW ($RT1^l$), BN ($RT1^n$), and ACI ($RT1^{av1}$) rats, 10–16 weeks of age, were purchased commercially from Harlan Sprague-Dawley (Indianapolis, IN). All animals brought into the experimental colony were certified virus-free, and the colony is monitored regularly for accidental contamination with infectious diseases. The animals were housed in microisolator cages in isolated animal facilities. The animals were fed rodent laboratory chow (Purina Mills, Inc., St. Louis, MO) and tap water ad libitum. The animal facilities at Cedars-Sinai Medical Center are accredited by the American Association for Accreditation of Laboratory Animal Care, and the animals included in these studies were handled humanely in accordance with animal experimental protocols approved by the Institutional Animal Care and Use Committee.

Mice: The mice used for the contact sensitivity experiments were Balb/c females purchased from Charles River Breeding Laboratories (Kingston, MA). All mice used in these experiments were 8–10 weeks of age and were maintained at the DuPont Merck Pharmaceutical Company Experimental Station, Wilmington, DE.

Heterotopic cardiac grafts. Abdominal grafts: Intraabdominal heterotopic cardiac grafting was performed using a modification of the technique described by Ono and Lindsey (*9*). The donor animals were anesthetized with ketamine (100 mg/kg), xylazine (10 mg/kg), and atropine (0.05 mg/kg) administered intraperitoneally, and then maintained as necessary on methoxyflurane via inhalation. The venae cavae and the pulmonary veins were ligated with 5-0 silk, and the pulmonary artery and aorta were transected 2–3 mm above their origins in the heart. After perfusion of the ventricles and atria with lactated Ringer's solution (containing 200 U/ml of heparin), the heart was placed in a saline bath at 4°C. Recipient animals were anesthetized as described above, a midline incision was made, and the great abdominal vessels were dissected free from the left renal vein to the bifurcation. The graft was implanted in the abdominal cavity with end-to-side anastomoses of the donor to recipient aortas and of the pulmonary artery to recipient vena cava in a running continuous suture with 10-0 Novafil on a TE-70 needle. Operative times ranged from 30 to 45 min, with a success rate of approximately 90%. The grafts were evaluated for function by abdominal palpation and all grafts were removed for examination at the termination of the experiment. At removal the hearts were fixed in 10% buffered formalin for 24 hr and then stored for histological processing.

Cervical grafts: Heterotopic cardiac grafts placed in the subcutis of the neck were used for examining the specificity of tolerance induction in recipients of orthotopic liver grafts that had survived for more than 120 days. The grafts were harvested as described above and were placed in a cervical location in the recipient. The donor aorta and pulmonary arteries were anastomosed in a running end-to-side fashion with the recipient carotid artery and external jugular vein, respectively. The operative times ranged from 30 to 45 min, with a success rate of approximately 90%. The grafts were evaluated for function by cervical palpation and all grafts were removed for examination at the termination of the experiment.

Heterotopic kidney grafts. Donor operation: All surgical procedures were performed under inhalation anesthesia. The donor left or right kidney was exposed via a midline incision and 200 IU of heparin was administered intravenously. The renal artery and vein, aorta, and the inferior vena cava were dissected free. A polyethylene catheter was inserted into the aorta and the kidney was flushed with 10 ml of a preservation solution over a 2-min period. The kidney was excised, placed in a container of cold perfusate, and stored by hypothermic immersion at 4°C prior to transplantation.

Recipient operation: Heterotopic kidney transplantation was performed following dissection of the recipient abdominal vessels. The donor renal artery and vein were anastomosed to the recipient aorta and inferior vena cava, respectively, using a continuous 10-0 Novafil suture on a TE-70 needle. Circulation was restored and reconstruction of the donor ureter was completed with an end-to-end anastomosis with an absorbable suture material. A nephrectomy of the recipient kidneys was performed after the placement of the donor kidney and prior to closure of the abdomen.

Orthotopic liver grafts. Donor operation: The grafting techniques used were modified from those originally described by Kamada et al. (*10*). Male rats of 250–300 gm bodyweight were anesthetized with methoxyflurane and the liver was removed through a midline incision. The left inferior infraphrenic vein was separated from the superior vena cava, double ligated with 6-0 silk, and cut. The caudate lobes of the liver were freed to expose the hepatoesophageal plexus, which was then ligated and cut. The infrahepatic vena cava was freed from surrounding fatty tissue. The preparation of the portal area was commenced by double ligation and division of the pyloric vein. To obtain the celiac segment, the hepatic artery was separated from the gastroduodenal artery, which was double-ligated and divided. After ligation and division of the left gastric and splenic arteries, the hepatic artery was dissected to the celiac trunk. The bile duct was exposed and a stent, approximately 8 mm long, prepared from a 22-gauge i.v. catheter, was inserted half its length into the bile duct, and the bile duct was now completely cut. The liver was perfused with cold saline (4°C) through the portal vein, the right suprarenal vessels were transsected, and the liver was removed and stored in a 4°C cold saline bath.

Recipient operation: After removal of the recipient liver, the donor organ was positioned and the anastomosis of the SVC was performed. The animal was turned back into the normal position, the portal vein stumps were approximated with 8-0 angle sutures, and the venous blood supply of the liver was restored. The anhepatic time did not exceed 20 min. The infrahepatic inferior vena cava was reconnected with 8-0 nylon suture and the common hepatic artery was rearterialized according to the method described by Steffen et al. (*11*). A single 10-0 nylon stay suture was fixed at the margin of the arterial opening and a vascular cuff was prepared from a 24-gauge i.v. catheter. The donor celiac artery was pulled over the cuff and fixed with a 6-0 ligature. Arterial blood flow was then reestablished. The bile duct anastomosis was accomplished as described by Zimmermann et al. (*12*) by suturing the free margin of the donor bile duct to the recipient bile duct with 8-0 sutures. A two-layer suture of the abdominal wall and the skin with 3-0 absorbable suture was placed to complete the recipient operation. Successfully grafted rats recover and start drinking water in 1–2 hr.

Pharmacologic agents. Brequinar sodium: This agent was formulated fresh daily in distilled-deionized water at concentrations of 1, 2, and 6 mg/ml and administered orally by gavage three times weekly for inhibiting allograft rejection. All mice were treated with the drug prepared fresh daily in 0.25% methocel (Dow Chemical Co., Midland, MI).

Cyclosporine A: A commercially available formulation of CsA (100 mg/kg) from Sandoz Pharmaceutical Corporation (East Hanover, NJ) was diluted in olive oil and administered orally by gavage at a dosage of 1 mg/kg three times weekly. For the contact sensitivity experiments, CsA was administered daily.

Mixed lymphocyte reaction. The sensitivity of one-way rat mixed lymphocyte responses to inhibition with BQR was tested by incubation of purified ACI and LEW splenic lymphocytes with increasing concentrations of the drug. A total of 2×10^5 responder lymphocytes/well were cultured with 2×10^5 irradiated donor, third-party, or syngeneic lymph node cells for 5 days in 5% CO_2 at 37°C. The culture medium consisted of RPMI 1640 medium (GIBCO, Grand Island, NY) containing 5% pooled sterile ACI serum, 2 mM l-glutamine, 1×10^{-5} M 2-mercaptoethanol, 0.1% nonessential amino acids, 1 mM Na pyruvate, and penicillin/streptomycin. Sixteen hr prior to harvesting, the cultures were pulsed with 1 μCi of ^{3}H-thymidine. At harvesting the cultures were collected on glass fiber mats and the level of thymidine incorporation were measured by counting in a beta scintillation counter.

Contact sensitivity and related methods. Mice were sensitized by application of 25 μl of 0.5% 2,4-dinitrofluorobenzene (DNFB; Eastman

Kodak Company, Rochester, NY) in a vehicle of consisting of a 4:1 solution of acetone and olive oil to the shaved abdominal skin on days 0 and 1. On day 5, the mice were challenged by application to ear skin of 20 μl of 0.2% DNFB. The ears were measured for thickness with a micrometer immediately before challenge and 24 hr later. The difference in ear thickness is expressed units of $10^{-4} \pm 1$ SE.

Statistics. The effect of BQR on the survival of the allografts was estimated using Kaplan-Meier curves. The differences in survival between different groups were assessed using the log-rank test (generalized Savage/Mantel Cox). Procedures involving the comparison of experimental data from groups of individual animals first had the equality of variance examined using the F test (two groups) or Levene's test (multiple groups). When normally distributed sample means were compared, the Student's *t* test (two groups) was used. Differences between test groups were considered to be statistically significant when $P<0.01$.

RESULTS

Suppression of mixed lymphocyte responses. The incubation of increasing concentrations of BQR in MLR cultures between ACI and LEW rats was associated with a sharp inhibition of the proliferative reaction (IC_{50} = 150 ng/ml) (Fig. 1). The BQR was added to the cultures at the beginning of the experiment and was present throughout the entire culture period. We have previously demonstrated in comparable experiments that the IC_{50} for CsA in rats is 45 μg/ml and that for FK506 the IC_{50} is 0.33 ng/ml (*13*).

Inhibition of contact sensitivity. The contact sensitivity response to DNFB is a delayed-type hypersensitivity reaction. As such, BQR was used to determine the effect of the drug on this in vivo T-lymphocyte–mediated immune response. The activity of BQR was compared with that of CsA, an effective immunosuppressive drug that can inhibit T-lymphocyte function in this assay. The results demonstrate that Brequinar is more potent (ED_{50} = 0.5 mg/kg) in this model than CsA (ED_{50} = 60 mg/kg) when the two drugs are administered orally from day 0 through day 6 (Table 1).

Heterotopic cardiac allografts. Abdominal heterotopic cardiac allografts were exchanged between ACI donor and LEW recipient animals and the recipients were treated with BQR. The treatment at each dosage level began 1 day prior to transplantation and continued until 30 days after surgery. Administration of BQR in increasing doses was associated with prolongation of median graft survival at all levels of the drug tested (Table 2). Median graft survival was significantly prolonged ($P<0.002$) at the 12 and 24 mg/kg treatment levels. The selection of a treatment schedule of three times weekly was based on observations in humans suggesting that this was the most effective dosage schedule for the antineoplastic effect of the drug. At the higher dosage levels (24 mg/kg), all of the recipient grafts survived the treatment period. Once treatment had stopped, the grafts were rejected in approximately 2 weeks.

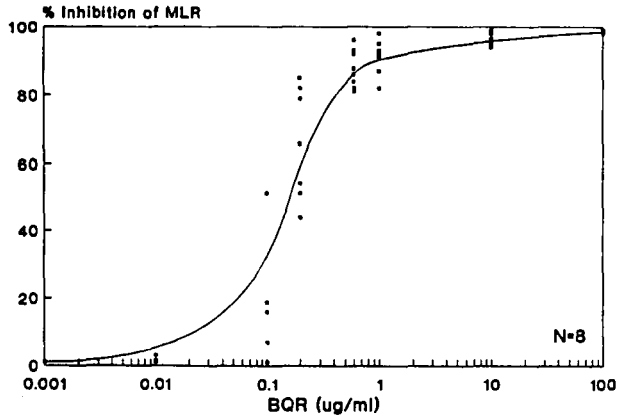

FIGURE 1. The inhibition of one-way mixed lymphocyte responses between ACI and LEW strain rats by Brequinar sodium. The data represent the inhibition of MLR in eight animals in three different experiments.

TABLE 1. The inhibition of the contact sensitivity response to DNFB in mice by Brequinar sodium and cyclosporine A[a]

Treatment group	Dose (mg/kg)	Change in ear swelling	% Suppression[b]	ED_{50}
Vehicle control	—	1.8±0.8	—	—
Positive control	—	56.8±3.0	—	—
CsA	2.0	49.2±3.8	13.9	
	10.0	44.9±3.4	21.7	
	50.0	41.8±3.2	27.3	60
	100.0	11.7±2.5	82.0	
BQR	0.4	32.3±3.2	44.7	
	2.0	16.9±3.2	72.7	0.5
	10.0	1.8±1.1	100.0	
	20.0	4.6±1.4	94.9	

[a] Balb/c mice were sensitized with 0.5% DNFB on days 0 and 1. On day 5, the ears were measured and then challenged with 0.2% DNFB. The increase in ear thickness was measured 24 hr following challenge.

[b] $$\% \text{ Suppression} = \frac{(\text{positive control} - \text{vehicle control})}{(\text{positive control} - \text{vehicle control})} \times 100$$

TABLE 2. The effect of Brequinar sodium on cardiac allograft survival in rats: ACI→Lewis

Drug treatment group[a]	n	Graft survival time (days)	Median survival (days ± 1 SD)	P^b
None	11	5,6,6,6,7×7	7.0±0.69	—
6 mg/kg	6	8,13,15,15,16,16	15.0±3.06	0.002
12 mg/kg	8	14,38,45,45,46,47,49,54	45.5±12.26	0.002
24 mg/kg	7	12,42,42,43,43,43,49	43.0±12.21	0.001

[a] BQR administered p.o. ×3/week, days −1 → +30.
[b] Compared with the control group.

Orthotopic liver allografts. The administration of BQR to the recipients of orthotopic liver grafts was also associated with significant improvement in graft survival (Table 3). The graft survival induced by treatment with 12 mg/kg of BQR was frequently permanent, with approximately 50% of the grafts surviving greater than 250 days. In contrast to the heart graft recipients, administration of BQR at levels of 24 mg/kg was associated with a sharply increased mortality in the graft recipients. These animals died with signs of drug toxicity, primarily diarrhea, suggesting that the liver transplantation procedure may have been responsible for a decreased ability to excrete the drug. The clinical signs of toxicity were associated with histopathological evidence of necrosis and atrophy of the gut epithelium in the intestinal crypts and hypocellularity of the bone marrow (data not shown).

The effectiveness of BQR when used in suboptimal doses

TABLE 3. The effect of Brequinar sodium on liver allograft survival in rats: ACI → Lewis

Drug treatment group[a]	n	Graft survival time (days)	Median survival (days ± 1 SD)	P^b
Control	8	9,10,10,10, 10,10,11,12	10.0±0.9	—
6 mg/kg	8	7,8,9,9,18, 29,69,>250	13.5±83.5	0.36
12 mg/kg	26	6,6,7,8,8,13, 14,15,19,19, 23,26,44, 139,12 × >250	91.5±117.2	0.008
24 mg/kg	8	6,6,6,7,11, 18,23,23	9.0±7.7	0.49
CsA 1 mg/kg	8	9,10,10,10, 10, 10, 11, >230	10.0±77.8	0.70
BQR 6 mg/kg + CsA 1 mg/kg	8	7,7,10,37, >220,>220, >221, >222	220.6±110.3	0.09

[a] BQR and CsA administered p.o. ×3/week, days −1 → +30.
[b] Compared with the control group.

TABLE 4. The effect of Brequinar sodium on kidney allograft survival in rats: ACI→Lewis

Drug treatment group[a]	n	Graft survival time (days)	Median survival (days ± 1 SD)	P^b
Control	9	5,6,6,6,6,6,6,6,6	6.0±0.3	—
12 mg/kg	6	>87,>93,>93, >99,>99, >99	>99.0±4.90	0.003
24 mg/kg	9	7,7,10,11,13,15, 18,29,>92	13.0±26.95	0.006

[a] BQR administered p.o. ×3/week, days −1 → +30.
[b] Compared with the control group.

TABLE 5. Induction of specific tolerance to cardiac grafts in the recipients of long-term surviving liver allografts

Recipient strain	n	Liver donor strain	Heart donor strain	Median graft survival (days)
LEW	4	ACI	ACI	>160 days
LEW	4	ACI	BN	8.00±0.50

with suboptimal doses of CsA was examined in recipients of orthotopic liver grafts. Small doses of CsA (1 mg/kg three times weekly) and BQR (6 mg/kg three times weekly) that separately do not induce significant prolongation of graft survival were administered alone and in combination to liver recipients for a period of 30 days (Table 3). The combination of the two drugs was highly effective in prolonging graft survival to a median of greater than 200 days in a small group of animals.

Heterotopic kidney allografts. Treatment of kidney allograft recipients at 12 mg/kg three times weekly was also associated with excellent graft survival (Table 4). All of the recipients in this group had permanent survival of their kidney allografts, with survival times in excess of 90 days. The kidney graft recipients treated with 24 mg/kg of BQR displayed, as seen with the liver recipients, an increased sensitivity to the toxic side effects of the drug. The median survival for the animals in this group was 13 days posttransplantation.

Specificity of the allograft tolerance. The nature of the tolerance induced by BQR in the recipients of orthotopic liver grafts was examined in animals that had received their liver grafts more than 120 days earlier. Small groups of these animals received a heterotopic cervical cardiac graft from the original donor strain (ACI) or an unrelated third-party strain (BN). Animals that received donor-strain hearts permanently accepted the heart grafts. The third-party hearts were rejected at times comparable to those expected for first-set rejection reactions (Table 5).

DISCUSSION

Recent advances in immunosuppressive therapy have allowed for the development of organ transplantation as an important therapeutic procedure. The most powerful of these new immunosuppressive drugs, cyclosporine A, is responsible for graft survival rates for many organs that approach 90% for the first posttransplant year. These successes, however, are associated with limitations in the total amount of CsA that can be administered because of toxic side effects, individual patients who fail to respond to CsA therapy and require alternative drug treatment, and the lack of effectiveness of CsA in preventing B-lymphocyte–mediated antibody production. In an attempt to provide alternatives for these limitations, we have examined the effectiveness of a new immunosuppressive drug, Brequinar sodium, for the prevention of transplantation rejection.

Our results demonstrate that BQR is an effective immunosuppressive agent. The compound acts to inhibit dihydroorotate dehydrogenase (DHO-DH), an effect that results in a decrease of de novo pyrimidine biosynthesis and a reduction in the nucleotide pool available for cell replication. Normal lymphocytes contain relatively low levels of DHO-DH and activated, proliferating lymphocytes are very sensitive to the effects of BQR (*8*). BQR is, as an example, a potent suppressor of the contact sensitivity response to DNFB in mice. These results suggest that BQR acts on the sensitization limb of this T-lymphocyte–mediated reaction, the portion of the response that is proliferation-dependent. The immunosuppressive activity of BQR extends to rats, as the drug exhibits a highly effective inhibition of the in vitro MLR between ACI and LEW rats.

The experiments described above demonstrate that BQR is highly effective in preventing the rejection of a variety of vascularized organ grafts. Treatment of LEW recipient rats with BQR can provide a high level of protection from rejection of ACI strain heart, kidney, and liver grafts. The recipients of heterotopic heart grafts display good graft survival at drug levels of 12 to 24 mg (administered three times weekly) for as long as the treatment is continued. Once the treatment ceases, the heart grafts are rejected approximately 2 weeks later, indicating that this period of treatment and graft acceptance is not sufficient to induce long-term tolerance to the graft. The recipients of both kidney and liver grafts also are responsive to treatment with BQR, with induction of long-term graft survival after treatment with the drug at a rate of 12 mg/kg. The recipients of these grafts display some important differences when compared with the heart recipients. The administration of 24 mg/kg to the kidney and liver recipients results in a dramatic decrease in the median survival time of the recipients and the onset of clinical and pathological signs of drug toxicity. Approximately 65–70% of the drug and its metabolites are

cleared via the bile and feces and 25–30% in the urine. The animals in the high-dose treatment group appear to be more sensitive to bone marrow and gastrointestinal side effects of the drug, perhaps due to temporary impairment of liver and kidney function following transplantation or to the interference with drug metabolism by high circulating levels of the drug.

The treatment of liver and kidney graft recipients with the drug for a period of 30 days is sufficient to induce a permanent, stable tolerance to the graft. Approximately 50% of the liver graft recipients and all of the kidney graft animals treated with 12 mg/kg exhibit long-term graft survival that has extended for more than 90 to 250 days. The tolerance induced to liver grafts is specific for donor tissues. Recipients of liver grafts that had survived for more than 120 days were challenged with heart grafts from either the donor strain or an unrelated third-party BN strain. The third-party grafts were rejected promptly, while hearts from the original donor strain have remained functional for more than 160 days. We have previously observed a similar difference in the ease of inducing tolerance to liver grafts (when compared with heart grafts) in studies conducted with FK506, suggesting that this phenomenon is the result of differences in the organ graft, rather than a feature of the immunosuppressive activity of BQR (*14*, *15*).

In summary, our experimental results have demonstrated that Brequinar sodium can induce effective suppression of normal immune responses, including MLR in the rat and the delayed hypersensitivity reaction seen in skin contact sensitivity to DNFB in mice. This suppression of normal immune responsiveness extends to the prolongation of vascularized organ allografts in rats. Treatment with BQR prolongs the survival of heart, kidney, and liver allografts in LEW strain recipients, with permanent graft survival in the majority of kidney and liver graft recipients when treated with the most effective doses of BQR.

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ORAL DISCUSSION

DR. BOLLINGER (Durham, NC): You have added to the potpourri of new immunosuppressive drugs. One of the challenges that has yet to be addressed is small intestinal transplantation. The idea that you could transplant the liver, and achieve indefinite tolerance of the heart suggests that perhaps you might do the same with the intestine. Do you have any data on gut transplantation either alone or with the liver transplant using Brequinar?

DR. CRAMER: No, we have done small bowel transplantation with other compounds, but not yet with Brequinar. Part of our reluctance has been that one of the major target organs for Brequinar is the gut.

DR. KAHAN: I was very impressed with the low dose level for mouse and human compared with the high rat dose of 12 mg/kg, yet you had a high bioavailability. It almost seems like the drug is more effective with mouse and human cells than it is with rat cells, possibly the reverse of cyclosporine. How do you explain the fact that you needed such a high dose of drug through the oral route if you had a high oral bioavailability? Did you do in vitro studies with rat cells to see whether or not they were as sensitive to Brequinar as mouse and human cells?

DR. CRAMER: Yes. A series of experiments looking at the effect of Brequinar on MLR proliferation have been completed. There was a wide range in sensitivity between species. The most effective inhibition was with rodents, humans, and monkeys. There was less effectiveness when pigs and dogs were examined. We think the relationship between in vitro sensitivity and graft survival may correlate.

DR. VAN BUREN (Houston, TX): Can you elaborate on the drug's toxicity? You indicated that one rapidly dividing group of cells, namely, the gut, is potentially a target tissue. Is this drug myelosuppressive? Can you give us some idea of the window between the toxic and therapeutic doses?

DR. CRAMER: The toxic side effects were very consistent from species to species. We have done pharmacotoxicity work in a variety of species. Almost all species exhibited bone marrow and gut effects. There was a fair difference in susceptibility to the drug between species. Dogs are very sensitive, whereas monkeys and humans tend to be relatively insensitive. At this point, I can't give you an exact correlation between toxic plasma levels and effectiveness of the drug for allograft survival. In rats, however, treatment with 12 mg/kg three times a week resulted in plasma levels of about 3–4 μg/ml. That level resulted in prolonged graft survival.

REFERENCES

1. Borel JF, Feurer C, Magnee C, Stahelin H. Effects of the antilymphocyte peptide cyclosporin-A in animals. Immunology 1977; 32: 1017.
2. The Canadian Multicenter Transplant Study Group. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. N Engl J Med 1983; 309: 809.
3. Thompson AW. FK-506: profile of an important new immunosuppressant. Transplant Rev 1990; 4: 1.
4. Borel JF, Kis ZL. The discovery and development of cyclosporine (Sandimmune). Transplant Proc 1991; 23: 1867.
5. Bennett WM, Pulliam JP. Cyclosporine nephrotoxicity. Ann Intern Med 1983; 99: 851.
6. Meyers BD, Ross J, Newton L, Luetscher J, Perlroth M. Cyclosporine-associated chronic nephropathy. N Engl J Med 1984; 311: 699.
7. Chen SF, Ruben RL, Dexter DL. Mechanism of action of the novel anticancer agent 6-fluoro-2-(2′-fluoro-1,1′-biphenyl-4-yl)-3-methyl-4-quinolinecarboxylic acid sodium salt (NSC 368390): inhibition of de novo pyrimidine nucleotide biosynthesis. Cancer Res 1986; 46: 5014.
8. Jaffee BD, Jones EA, Chen S-F, Cramer DV, Makowka L. Brequinar sodium is a novel immunosuppressive agent with a unique mechanism of action. Proceedings of the 10th Annual Meeting of the American Society of Transplant Physicians [Abstract], 1991: 270.
9. Ono K, Lindsey ES. Improved technique of heart transplantation in rats. J Thorac Cardiovasc Surg 1969; 57: 225.
10. Kamada N, Calne RY. Orthotopic liver transplantation in the rat—technique using cuff for portal vein anastomosis and biliary drainage. Transplantation 1979; 28: 47.
11. Steffen R, Ferguson DM, Krom RAF. A new method for orthotopic rat liver transplantation with arterial cuff anastomosis to the recipient common hepatic artery. Transplantation 1989; 48: 166.
12. Zimmermann FA, Butcher GW, Davies HS, Brons G, Kamada N, Tuerel O. Techniques for orthotopic liver transplantation in the rat and some studies of the immunologic responses to fully allogeneic liver grafts. Transplant Proc 1979; 11: 571.
13. Eiras G, Imventarza O, Murase N, et al. Species differences in sensitivity of T lymphocytes to immunosuppressive effects of

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Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

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Sync your system to PACER to automate legal marketing.