Gastrointestinal Decontamination of Dogs Treated with Total Body Irradiation and Bone Marrow Transplantation

Huib M. Vriesendorp, Peter J. Heidt & Chris Zurcher

Radiobiological Institute TNO, Institute for Experimental Gerontology TNO, P.O. Box 5815, 2280 HV Rijswijk, The Netherlands

Received 13 August 1980, accepted 19 June 1981

Procedures for total and selective gastrointestinal decontamination of dogs are described. The selective procedure removed only Gram negative aerobic bacteria, yeast and fungi. Dogs receiving total decontamination were less susceptible to the GI syndrome following total body irradiation (TBI) than dogs receiving conventional care. After TBI and allogeneic bone marrow transplantation, serum albumin levels contaminated animals. Endogenous infections after total body irradiation were prevented only by total decontamination. Endogenous infections occurred in selectively decontaminated animals, but with milder clinical symptoms than in conventional animals. Appearance of donor type leukocytes and serum gamma globulin was slower in decontaminated animals than in conventionally treated controls. Acute graft versus host disease caused by a limited number of lymphocytes of a DLA identical littermate donor were prevented by selective gastrointestinal decontamination. Complications due to late immune reconstitution obscured the effect of decontamination on delayed graft versus host disease.

Keywords: total body irradiation – bone marrow transplantation – gastrointestinal decontamination

Allogeneic bone marrow transplantation can be a life saving procedure for human patients (1–3). The application is limited by its inherent immunobiological complications—the host versus graft and the graft versus host reactions. The major histocompatibility complex (MHC) has an important influence on the outcome; by use of MHC identical sibling bone marrow the host versus graft reaction can be controlled by total body irradiation (TBI) (4), and the incidence of lethal graft versus host disease (GvHD) can be decreased.

Studies in mice have shown that lethal GvHD can be prevented if recipients of allogeneic MHC mismatched bone marrow cells are raised and kept under germfree conditions (5–7). Lethal GvHD is also prevented in conventional mice freed of their gastrointestinal microorganisms by orally administered nonabsorbable antibiotics (6,7), a procedure known as total decontamination. Removal of the aerobic gastrointestinal bacterial microflora allowing anaerobes to remain, is called selective gastrointestinal decontamination. This procedure provides effective GvH prevention in mice (7). Anaerobic microflora in the GI tract makes animals less susceptible to colonizations with exogenous microorganisms (8). Studies in rhesus monkeys (9) and in a small group of human patients (10) have shown a beneficial effect of gastrointestinal decontamination on GvHD.

This report is on a study of the effect of gastrointestinal decontamination (GD) on GvHD in dogs. In addition, an analysis is made of the effects of GD on (1) endogenous and exogenous infections, (2) susceptibility to gastrointestinal radiation damage, and (3) regeneration rate of the hemopoietic system after transplantation.

MATERIALS AND METHODS

Dogs. Recipients and donors were beagles of either sex purchased from the Central Breeding Colony for Experimental Animals of TNO (Zeist, The Netherlands). Their weight ranged from 7.5 to 15.0 kg and their ages 0.5–5 years. They were immunized against hepatitis, distemper and leprosy.

Genetics. The major histocompatibility complex of the dog (DLA) was analyzed for serologically defined and lymphocyte defined determinants (11,12). DLA identical combinations reported in this paper were always serologically identical as well as negative in reciprocal unilateral mixed lymphocyte culture responses. Genetic markers used to identify donor BM cells after grafting were sex chromosomes and the polymorphic blood cell enzymes phosphoglucomutase 2 (PGM 2), Peptide D (PepD) and Superoxide dismutase-soluble form (SOD-s). Determinations of enzyme phenotypes were performed by Dr. P. Meera Khan of Leiden University, The Netherlands, following described procedures (13).

Total body irradiation (TBI). Radiation dosimetry and biological response to the radiation protocol used are described (14), briefly, two opposing Philips-Muller X-ray machines were used. Physical parameters were 300 kV, 10 mA, HVL 3.0 mm Cu and an average of 0.16 Gy/min. Animals were confined in a close fitting aerated wooden box during radiation without anaesthesia.

Cell suspensions. Bone marrow cells were given 6–30 h after TBI completion. Sometimes lymph node cells, from the bone marrow donor, were added to the transplant to obtain a better approximation of the observed incidence and severity of GvHD in human bone marrow transplantation (14). Bone marrow cell suspensions were obtained by one of two methods: 1) aspiration of the humeri or femora or both under general anesthesia with ketamine chloride and acepromazine. Cells were collected in bottles containing Hanks' Balanced Salt Solution. DNAse and heparin; 2) exsanguination of the donor followed by processing of bones containing red marrow, using a high pressure bone press, with collection of cells as under 1). After centrifugation at 1000 g for 10 min, fat was removed from the suspensions. Buffy coat cells were collected, filtered through six layers of autoclaved gauze to remove remaining bone particles, and counted. The cell count was corrected for dead cells. The cells were administered to the recipient via a conventional i.v. set.

Lymph nodes were obtained by aseptic surgical removal. After cutting the nodes into approximatively 1–2 mm3 fragments, these fragments were pressed through six layers of autoclaved gauze with a plunger of a syringe to obtain a monocellular suspension. Cells were washed
once in Hanks’ Balanced Salt Solution prior to counting.

Animal care. The dogs were studied under three animal care conditions: conventional, selective gastrointestinal decontamination and total gastrointestinal decontamination. The procedures used in conventional care have been described (14). Briefly, animals were housed in individual cages and dewormed prior to irradiation. A low percentage of dogs were carriers of *Pseudomonas* species in their stool on arrival in the laboratory. These dogs were irradiated after these microorganisms had disappeared in all of them under oral colistin treatment. After irradiation, the animals received acidified water (pH 3), and sterile food. Rectal temperature and fluid intake and output were recorded daily. Parenteral fluid or antibiotics, and whole blood or platelet transfusions, exposed to 15 Gy, were given when clinically indicated. From day 3 to day 7 after TBI, the dogs received no oral fluids, but were administered 500 ml of Ringer lactate s.c. twice daily. Antibiotics were administered if rectal temperatures rose above 39.7°C. The least expensive combination of two antimicrobial agents, expected to be effective was chosen on the basis of the fecal antibiogram obtained prior to TBI and repeated weekly; simultaneous, prophylactic antifungal therapy (160 mg pimafucin p.o. twice a day) was initiated. The antibiogram, using disks impregnated with ampicillin, carbenicillin, cephalothin, colistin, streptomycin, kanamycin and tobramycin, was done on 5% sheep blood agar. In all dogs, the least expensive combination of two antimicrobial agents was chosen on the basis of prior experience (15). Parenteral antibiotics were stopped five days after normalization of the rectal temperature, Oral pimafucin was continued for an additional three days.

Reverse isolation was provided for animals subjected to gastrointestinal decontamination. For the selective-GD dogs, hepa-filtered sterile air was ventilated through the cage at approximately 0.01 m/sec. This resulted in cage volume changes of about 40%. For total GD dogs, laminar airflow conditions were achieved by removing the cage roof and placing the cages under a laminar downflow ceiling and maintaining an airflow of 0.45 m/sec. The complexity of antimicrobial treatment and the bacteriologic surveillance were increased in the GD procedures. Oral antimicrobial agents, listed in Table 1, selected on the basis of prior experience (15) were administered twice a day. An uninterrupted period of at least five days of stable selective or total decontamination was allowed before the decision was made to irradiate the animals.

In selective-GD dogs, parenteral and orally administered antimicrobial agents were given when a rectal temperature above 39.7°C was recorded. The antibiogram of the fecal flora in these animals was made under both aerobic and anaerobic conditions. In total-GD dogs parenteral antimicrobial agents were administered only when exogenous infections were documented by microbiological techniques, without regard to rectal temperature.

A summary of the differences among the three treatment groups is given in Table 1.

**Termination of decontamination procedure.** Decontamination was discontinued in surviving animals on day 40 after irradiation (7). Oral pimafucin was continued for an additional week. Selective-GD animals were returned to conventional animal rooms and provided nonsterile food and nonacidified drinking water. Gram negative aerobic microorganisms gradually reappeared with return to normal fecal concentrations within one week. The fecal flora and total-GD animals remained in the lamaric air flow, while the other reverse isolation procedures were discontinued. Dogs were returned to conventional rooms when Gram positive aerobic or anaerobic microorganisms were no longer found in the feces. To accomplish this, some of the total-GD animals were fed fecal microflora from selective-GD dogs. In others, recolonizations occurred with exogenous microorganisms or with endogenous microflora that had been suppressed, but not eliminated during oral antimicrobial treatment.

**Experimental groups.** In DLA identical donors, recipient pairs the addition of lymph node cells is required to enhance GvHD to a severity that is comparable to that observed in primates (14). Studies on the genetic control of GvHD observed in these dogs is being reported elsewhere (16). The conventional bacteriologic treatment was evaluated in 33 dogs treated with 7.5 Gy and 2 x 10⁸ bone marrow cells/kg body weight and 4 x 10⁸ conventionally treated dogs that received 10¹⁰ autologous bone marrow cells/kg after 9.0 Gy (2) or 8.5 Gy TBI (2). The DLA mismatched bone marrow cells were rejected therefore those animals will be considered only for the effect of GD on the GvH syndrome and exogenous infections. The different experimental protocols were performed concurrently and completed over a four year period.

**Bacteriologic sampling and determinations.** Swab samples were taken from the oral cavity, skin and cages of the decontaminated dogs, at the frequencies indicated in Table 1. The swabs were moistened with and incubated in Brain Heart Infusion broth (BHI broth, Oxoid). Or fera were semiquantitated by streaking the swab onto a BHI agar plate. The rectal flora were quantitated by streaking the swab onto a BHI agar plate. The fecal concentration of bacterial species was determined by suspend­ ing approximately 0.1 g of fresh feces in a plastic tray containing 0.5 ml of BHI broth. The sus­ pension was then serially diluted 1:10 through 8 to 10 dilution steps. After overnight incubation at 37°C, subcultures from each cup were made on Endo agar (Oxoid), 7.5 Gy (2) and total-GD animals. Totally decontaminated animals were returned to conventional rooms when exogenous infections were documented by suspending approximately 0.1 g of fresh feces in a plastic tray containing 0.5 ml of BHI broth. The sus­ pension was then serially diluted 1:10 through 8 to 10 dilution steps. After overnight incubation at 37°C, subcultures from each cup were made on Endo agar (Oxoid), 40% of the survivors, at least three of the four examinations had to give positive results.

**RESULTS**

**Antimicrobial treatment after irradiation**

**General effects.** Major organ toxicity was not observed as a result of the intramuscularly (ampicillin, streptomycin, cephalothin, kanamycin, tobramycin), or orally administered (polymycin B, neomycin, cephalothin, pimafucin) antibiotics used in this study. Histological abnormalities in autopsies or biochemical deviations in surviving animals were related to radiation toxicity, infections or hemorrhages inherent to the bone marrow transplantation protocol used. There was no relation to the antibiotic regimens used. The only abnormal clinical symptoms attributed to the orally administered antibiotics were occasional vomiting and the occurrence of loose odorless stools in both selective GD and total GD animals. Intramuscularly administered antibiotics sometimes caused hemorrhages at the site of injection in thrombopenic animals. The size of these hemorrhages could be limited by appropriate platelet

**Transplants from 8 selective-GD dogs, and from 8 conventionally treated dogs that received DLA identical transplants. Polyacrylate strips (Cello­ gel) were used in a paper electrophoresis chamber of Gelman Instrument Company (1 h, 1.5 mA). Strips were stained with 0.2% Ponceau, washed 3 times with 5% acetic acid and measured in a chronoscan (Joyce).

**GvHD.** All animals that died during the study were subjected to a complete necropsy and histological analysis, diagnosis of GvHD was based on this histological analysis. A diagnosis of GvHD in surviving animals was based on: 1) clinical inspection of the animal; 2) histological analysis of monthly lymph node biopsies; 3) hematological signs; and 4) serum biochemistry as described (14,16). For the diagnosis of GvHD in the survivors, at least three of the four examinations had to give positive results.

**RESULTS**

**Antimicrobial treatment after irradiation**

**General effects.** Major organ toxicity was not observed as a result of the intramuscularly (ampicillin, streptomycin, cephalothin, kanamycin, tobramycin), or orally administered (polymycin B, neomycin, cephalothin, pimafucin) antibiotics used in this study. Histological abnormalities in autopsies or biochemical deviations in surviving animals were related to radiation toxicity, infections or hemorrhages inherent to the bone marrow transplantation protocol used. There was no relation to the antibiotic regimens used. The only abnormal clinical symptoms attributed to the orally administered antibiotics were occasional vomiting and the occurrence of loose odorless stools in both selective GD and total GD animals. Intramuscularly administered antibiotics sometimes caused hemorrhages at the site of injection in thrombopenic animals. The size of these hemorrhages could be limited by appropriate platelet
Sensitivity to the gastrointestinal syndrome. Dogs and negative cultures in total-GD dogs. That were bled daily over long periods of time. In conventional cases, collectively representative of the gastrointestinal microflora, i.e. gram positive aerobic microorganisms, with exogenous microorganisms not found during decontamination. Apparently, the oral antimicrobial treatment effectively removed Gram negative microorganisms, without induction of resistance. In the total-GD group, colonizations with exogenous microorganisms were looked for in animals that showed a bone marrow take and survived more than 20 days. Colonizations occurred in 5 of 7 animals (three autologous and four DLA identical littermate BM cells recipients), i.e. Pseudomonas aeruginosa in 3, a combined infection with Escherichia coli and Proteus mirabilis in a single animal, and yeasts in the remaining animal. Usually exogenous colonizations started in the oral cavity and were subsequently noted in feces. The infection with E. coli and P. mirabilis was the single exception, isolated only from a single exception, isolated only from a
Colonizations and endogenous infections after bone marrow transplantation in histocompatible donor recipient combinations

<table>
<thead>
<tr>
<th>Treatment recipient</th>
<th>Exogenous colonizations</th>
<th>Endogenous infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>frequent (see text)</td>
<td>in all recipients</td>
</tr>
<tr>
<td>Selective gut</td>
<td>not found (57 dogs, 370 treatment days)</td>
<td>in all recipients</td>
</tr>
<tr>
<td>Total gut</td>
<td>frequent (57 dogs, 370 treatment days)</td>
<td>not found</td>
</tr>
</tbody>
</table>

The second temperature peak observed in all animals (Fig 4) around day 18 after TBI cannot be explained. Blood cultures performed in all GI animals at the time of the second peak were negative. Without changing treatment, the elevated temperature gradually decreased.

Hematologic regeneration after bone marrow transplantation. In Fig 5, the leukocyte levels after TBI are shown for those animals in the different treatment groups which received cells from DLA identical littermate donors. Leukocyte recovery was slow in dogs with total-GD and rapid with conventional treatment. The 10^9/liter leukocyte level in selective-GD and total-GD animals occurred, respectively 2 and 5 days later than conventional animals (P < 0.05 in a Wilcoxon test).

At this stage of the bone marrow transplant procedure more than 90% of the leukocytes were granulocytes. No differences between conventional and GD animals were found in platelet or reticuloocyte recovery.

Initial serum gamma globulin levels after transplantation, shown in Fig 6, dropped in all groups approximately following the half-life of dog gamma globulin (3–4 weeks). Conventional animals subsequently showed an hypergamma globulinemia that returned to normal in the fifth month after irradiation.

GvHD incidence and survival. In Table 3, the incidence of GvHD is compared between the dogs in this study and our previous studies. Selective GD can apparently counteract the GvH promoting activities of 10^9 donor lymphocytes/kg body weight per recipient, however, in the small number of animals used, total-GD appeared to be insufficient to counteract the GvH promoting activities of 2 x 10^9 donor lymphocytes/kg body weight per recipient. The survival of the groups of Table 3 is depicted in Fig 7. Early GvHD mortality (within 60 days
after GD group. However, late mortality occurred in two animals (days 70 and 100), in which GvHD was not previously detected. In both dogs, histological analysis of necropsy material showed that the cause of death was necrotizing adrenalitis. Nuclear inclusion bodies suggestive of viral etiology were found. In contrast the delayed mortality in the animals showing rapid hemopoietic recovery, exogenous colonizations occurred (5 out of 7; one of them contributing to death of the animal). Of the animals in which DLA mismatched bone marrow cells failed to engraft permanently after TBI (n = 6, data not shown), only one dog could be kept totally decontaminated for twenty days after TBI, when it died from massive hemorrhages. The other 5 died before that time from complicating exogenous infections. The engraftment failures in the total GD dogs were caused by the DLA mismatches between donor and recipient. Under conventional conditions a similar high (almost 100%) engraftment failure rate has been reported (25). The absence of exogenous colonizations or infections in selective GD dogs indicated that the remaining endogenous flora in these animals effectively decreased the colonization rate of exogenous microorganisms. This supports the usefulness of the concept of colonization resistance as defined initially for mice (8).

Total GD appeared to have a beneficial effect on the acute GI syndrome after TBI. This effect might be related to the prevention of endogenous infections. At higher TBI doses, infections are no longer a contributory cause of death due to the GI syndrome since the animals die exclusively from fluid and electrolyte loss and total GD will no longer influence the outcome of the GI syndrome. In studies with conventional rats, infections appeared to contribute to the severity of the GI syndrome only at the lowest TBI dose that caused a lethal GI syndrome (26). Selective as well as total GD probably also have had an albumin sparing effect. A protein losing enteropathy has been described in mice suffering from GvHD (27). Albumin determinations in stool or urine were not performed in this study. The low albumin levels in the conventional dogs could also be caused by the more severe infections in this group and the concomitant longer period of negative nitrogen balance.

Endogenous infections were not seen in total GD dogs. They were observed in selective GD dogs, but with less se-

**DISCUSSION**

Selection of the kind and dose of oral antimicrobial agent for gastrointestinal decontamination was based on previous studies in dogs and rhesus monkeys (9,15). A deliberate choice was made for nonabsorbable drugs that do not give rise to important tissue concentrations after oral administration. Thus bone marrow reconstitution can proceed unhindered by the possible hemopoietic toxicity (nalidixic acid, trimethoprim sulfamethoxasole) (18,19), or other organ toxicity (e.g. liver toxicity of nalidixic acid) (26) of antimicrobial agents. Another advantage of the use of nonabsorbable drugs that do not give rise to complications is that it permits a different antimicrobial agent for gastrointestinal decontamination. Ly = lymph node cells. The experimental groups are the same as the ones listed in Table 3.

![Figure 6. Serum gamma globulin of dogs after bone marrow transplantation from a DLA identical donor.](image)

**Figure 6.** Serum gamma globulin of dogs after bone marrow transplantation from a DLA identical donor. Mean values, Standard deviations are given for selected points only to improve readability. The stippled area indicates the range of values in 10 normal dogs.

<table>
<thead>
<tr>
<th>Group</th>
<th>Bacteriological status</th>
<th>BM cells x10^6/kg</th>
<th>Ly cells x10^6/kg</th>
<th>Incidence of GvHD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>conventional</td>
<td>4-16</td>
<td>2</td>
<td>8/31</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>conventional</td>
<td>2</td>
<td>1</td>
<td>21/33</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>selective GD</td>
<td>2</td>
<td>1</td>
<td>3/11</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>conventional</td>
<td>2</td>
<td>3</td>
<td>12/12</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>total GD</td>
<td>2</td>
<td>2</td>
<td>4/4</td>
<td></td>
</tr>
</tbody>
</table>

* See Table 1 for details.

**Table 3**

Incidence of graft versus host disease in recipients of cells of a DLA identical littermate donor.
vere clinical symptoms in comparison to conventional animals. The lower endotoxin content of gastrointestinal microorganisms in the selective GD group might explain this difference as well as the slower regeneration rate of leukocyte and gamma globulin levels after TBI in these animals. Endotoxin is well known for its stimulating effects on hemopoietic progenitor cells as well as B-lymphocytes (28,29). The found is well known for its stimulating effects on leukocyte and gamma globulin levels

hypergammaglobulinaemia in the conventional dogs is similar to the one observed in long term surviving human patients with GvHD (30). Selective GD was found to mitigate acute GvHD in this study with dogs. In studies with mice in MHC mismatched donor-recipient combinations, the effects of decontamination were more pronounced on the delayed form of GvHD (7). The effect of GD on delayed GvHD could not be evaluated in dogs because late mortality occurred in conventional as well as selective GD animals. The conventional animals appear to die from immune deficiencies secondary to GvHD. Dogs that are similarly treated, but do not receive donor lymphocytes, show no delayed mortality (14). The underlying mechanism of late mortality in the selectively decontaminated animals would appear to be a primary immune deficiency (slow immunological recovery). The last part of this explanation is suggested by the slower reconstitution of leukocyte and gamma globulin levels in the decontaminated dogs as found in this study. A correction of the late immune deficiency would be required to reveal the complete beneficial effect of gastrointestinal decontamination on survival after allogeneic bone marrow transplantation. Perhaps this could be achieved by prolonging the period of gastrointestinal decontamination. A 40 day decontamination period was chosen for this study on the experience gained in mice (7), but might have been too short in dogs. Another possible approach to solve the immunodeficiency problem is the infusion of donor type lymphocytes towards the end of the first or second month after TBI. Studies using a longer interval (5 months after irradiation) have shown that this procedure does not necessarily cause lethal GvHD (31). Thus, the combined use of gastrointestinal decontamination and carefully applied stimulants of the donor immune system has attractive theoretical advantages but requires further animal experimentation. In this dog study, the impression is gained that the acute GvHD caused by 10^8 donor lymph node cells per kilogram body weight from a MHC identical donor is the maximum severity of GvHD that can be prevented by gastrointestinal decontamination. A more precise and reliable estimate of the maximum number of donor lymphocytes that can be counteracted by this procedure will require an analysis with larger numbers of dogs treated with different numbers of donor lymphocytes.

The selective GD used in this study offered important advantages over total decontamination. It is easier to perform and less risky than total GD. It prevents exogenous colonizations and infections, decreases the severity of endogenous infections and mitigates the severity of GvH disease. The infectious and GvHD problems of human bone marrow transplantation might become less severe under conditions of selective GD. However, selective GD has several inherent drawbacks such as low palatability of oral antimicrobial agents, costs of intensive bacteriological surveillance and slower immune recovery. This indicates the need for a controlled introduction of this technique comparing costs and benefits between conventional treatment and selective GD for a proper definition of the applicability of selective GD in human bone marrow transplantation.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Wim Klapwijk, Cees de Groot and Frits Vervat who executed the involved biotechnical and bacteriological procedures of canine gastrointestinal decontamination with admirable patience and efficiency.

REFERENCES


16. Vriesendorp HM, Frits Vervat who executed the involved biotechnical and bacteriological procedures of canine gastrointestinal decontamination with admirable patience and efficiency.
millan Publishing Co., Inc. N.Y., 1975;1007 & 1124.


Correspondence to:
H. M. Vriesendorp
Northwestern University Cancer Center
710 N. Fairbanks Court
Chicago, IL 60611, USA

ANNOUNCEMENT