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Jaebok Choi
Washington University School of Medicine in St. Louis

Matthew L. Cooper
Washington University School of Medicine in St. Louis

Edward D. Ziga
Washington University School of Medicine in St. Louis

Julie Ritchey
Washington University School of Medicine in St. Louis

John F. DiPersio
Washington University School of Medicine in St. Louis

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Brief Communication

Effect of Epigallocatechin-3-Gallate on Graft-Versus-Host Disease

Jaebok Choi,* Matthew L. Cooper,* Edward D. Ziga,†[1] Julie Ritchey,* and John F. DiPersio*

*Division of Oncology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA
†Division of Hematology/Oncology, Department of Pediatrics, Washington University School of Medicine, St. Louis, MO, USA

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is often complicated by alloreactive donor T-cell-mediated graft-versus-host disease (GvHD). The major polyphenol of green tea, epigallocatechin-3-gallate (EGCG), is an inhibitor of both DNA methyltransferase 1 (DNMT1) and signal transducer and activator of transcription 1 (STAT1), which are essential for induction of GvHD. Thus, in this report, we examine if in vivo administration of EGCG mitigates GvHD in several different animal models. While we concede that refinement of EGCG treatment might result in GvHD prevention, our results suggest that EGCG treatment might not be an effective therapy against GvHD in the clinic.

Key words: Graft-versus-host disease (GvHD); Allogeneic hematopoietic stem cell transplantation (allo-HSCT); Epigallocatechin-3-gallate (EGCG); DNA methyltransferase 1 (DNMT1); Interferon-γ receptor signaling; Signal transducer and activator of transcription 1 (STAT1)

INTRODUCTION

We recently reported that azacitidine (AzaC), a DNA methyltransferase 1 (DNMT1) inhibitor, and INCB018424, an inhibitor of janus kinase 1 (JAK1)/JAK2, which mediate interferon-γ receptor (IFN-γR) signaling via signal transducer and activator of transcription 1 (STAT1) phosphorylation, mitigate graft-versus-host disease (GvHD) while maintaining antileukemia effects (graft-versus-leukemia effect or GvL) after allo-hematopoietic stem cell transplantation (allo-HSCT) (1,2). The major polyphenol of green tea, epigallocatechin-3-gallate (EGCG), has been shown to be an inhibitor of both DNMT1 and STAT1 (4,8,9,13), thereby being a very attractive potential therapeutic agent to prevent or treat GvHD after allo-HSCT.

Hyon and colleagues have proposed that EGCG inhibits T-cell activation in vitro (7) and that EGCG-treated T-cells are less potent at inducing GvHD in vivo (5) acting through the blockade of stimulatory receptors. The authors' data have drawn attention to EGCG in the field of GvHD. Their in vitro proliferation analyses are both impressive and compelling. While EGCG-treated allogeneic splenocytes result in a significantly improved survival according to their most recent report, the benefit of EGCG in survival was minimal: median survival of 8 days (untreated) versus 10 days (EGCG) (5). Moreover, all the recipient mice died within 16 days of transplantation, perhaps due to the ineffectiveness of EGCG on blocking GvHD or partly because bone marrow cells were not transplanted along with splenocytes; thus, it is unclear whether the major cause of death was GvHD. In addition, the method used (in vitro incubation of purified donor C57BL/6 splenocytes incubated for 1 h at 4°C with 200 μM EGCG prior to infusion into Balb/c recipients) would be costly and time consuming when translated into the clinic. Therefore, in this report, we utilized a more clinically preferred method, that is, in vivo administration of EGCG into allogeneic recipients, to test if EGCG treatment results in reduced GvHD after allo-HSCT.

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[1]Current address: Blood & Marrow Transplant, University of Miami Miller School of Medicine, Miami, FL, USA.
Address correspondence to Jaebok Choi, Ph.D., Division of Oncology, Department of Medicine, Washington University School of Medicine, 660 S. Euclid Ave., Campus Box 8007, St. Louis, MO 63110, USA. Tel: +1-314-362-9349; Fax: +1-314-362-9333; E-mail: jchoi@dom.wustl.edu
or John F. DiPersio, M.D., Ph.D., Division of Oncology, Department of Medicine, Washington University School of Medicine, 660 S. Euclid Ave., Campus Box 8007, St. Louis, MO 63110, USA. Tel: +1-314-454-8491; Fax: +1-314-454-7551; E-mail: jdipersi@dom.wustl.edu

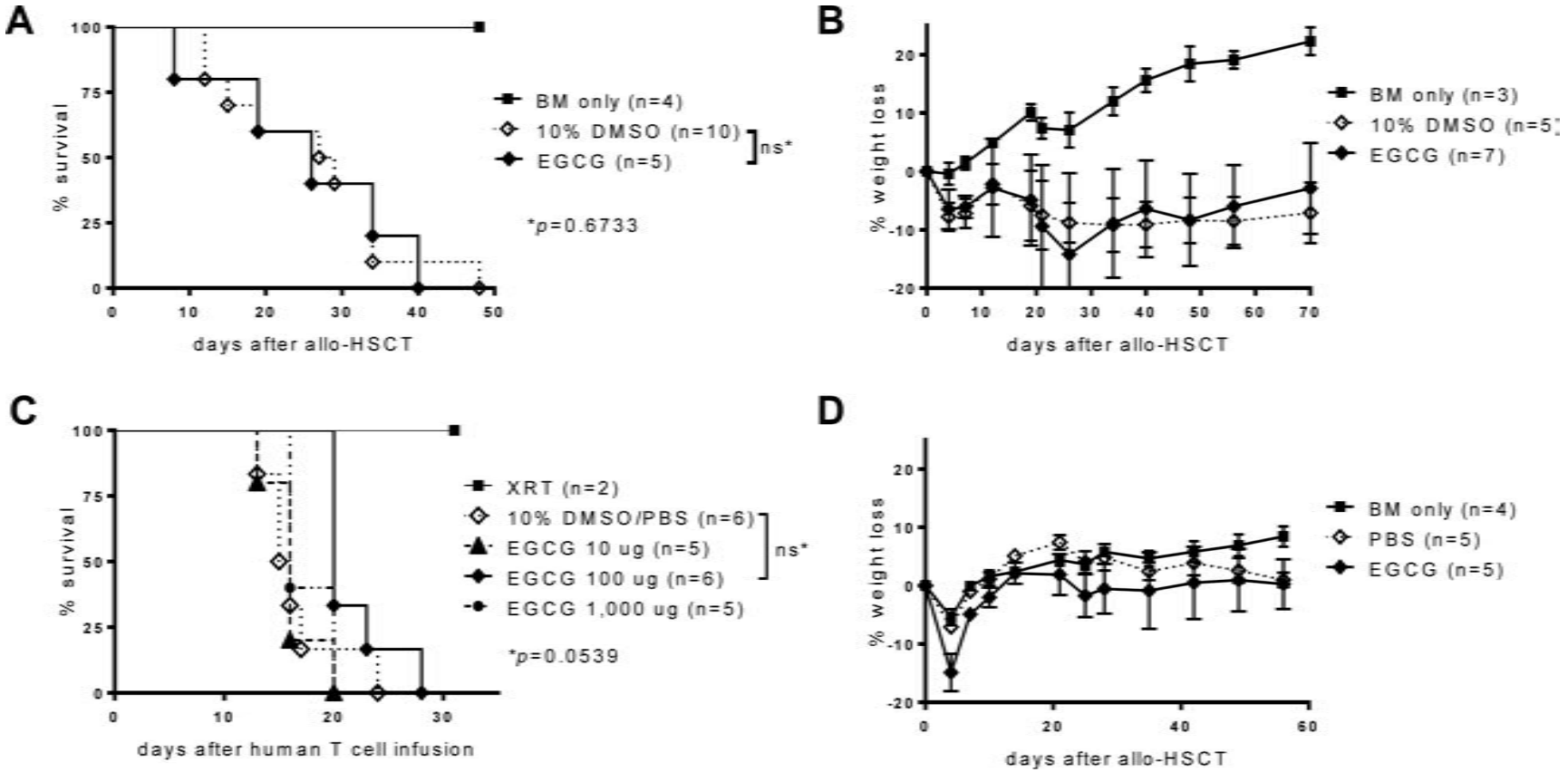

Figure 1. Effect of EGCG on GvHD. T-cell-depleted bone marrow (TCD BM) cells (5×10^6) [major histocompatibility complex class II H-2^b, cluster of differentiation 45.1 positive (CD45.1^+)] along with pan T-cells (H-2^b, CD45.2^+; 5×10^5 T-cells for Balb/c and 5×10^6 for C57BL/6×129) isolated from C57BL/6 donor mice were intravenously transplanted into Balb/c recipient mice (H-2^d, CD45.2^+) (A), which were lethally irradiated 1 day prior to the allo-hematopoietic stem cell transplantation (allo-HSCT) (925 cGy from a ^{137}Cs source), or into C57BL/6x129 F1 (1,200 cGy; H-2^b, CD229.1^+) (B). Epigallocatechin-3-gallate [EGCG; 100 μg in 10% dimethyl sulfoxide (DMSO) in phosphate-buffered saline (PBS)] was intraperitoneally (IP) injected twice a day for 21 days starting day 0 post-allo-HSCT. (C) Human pan T-cells (3×10^6) were retro-orbitally transplanted on day 0 into sublethally irradiated (250 cGy on day −1) nonobese diabetic/severe combined immunodeficient interleukin 2 (IL-2) receptor γ chain knockout (NSG) mice. EGCG (10–1,000 μg) was injected IP once every other day for 21 days starting day 3 post-human T-cell injection. XRT: xenotransplantation. Survival was compared using the log-rank test. (D) Donor mice (C57BL/6), not the recipients, were injected IP with EGCG (100 μg in PBS only) or carrier (PBS) each day for 4 days prior to T-cell harvest from donors. Donor splenocytes (2×10^7, CD229.2^+, CD45.2^+) and TCD BM (5×10^6, CD229.2^+, CD45.1^+) were injected via the lateral tail vein on day 0 into lethally irradiated C57BL/6x129 recipients (1,200 cGy on day −1; H-2^b, CD229.1^+).

MATERIALS AND METHODS

Mice

All mice (6- to 12-week-old males) were obtained from Jackson Laboratory (Bar Harbor, ME, USA). Animal study protocols including animal care and euthanasia were approved by the Washington University School of Medicine Animal Studies Committee.

Cells

Human peripheral blood mononuclear cells (PBMCs) were collected in compliance with the protocols outlined by the Washington University School of Medicine Human Studies Committee and harvested by Ficoll (GE Healthcare Bio-science AB, Uppsala, Sweden) gradient centrifugation. Human pan T-cells were isolated from the human PBMCs using Miltenyi microbeads and AutoMACS (Miltenyi Biotech, Auburn, CA, USA) (10). Mouse pan T-cells were isolated from mouse spleens using Miltenyi microbeads and an AutoMACS (Miltenyi Biotech) (10).

EGCG

EGCG was purchased from Sigma (St. Louis, MO, USA) and suspended in either 10% dimethyl sulfoxide (DMSO; Sigma)/phosphate-buffered saline (PBS; Sigma) or just PBS, for intraperitoneal injection. See figure legend for additional methods.

RESULTS AND DISCUSSION

We examined whether in vivo administration of EGCG, which is more clinically relevant than that of EGCG-treated cells, into allo-HSCT recipients reduces GvHD after allo-HSCT. Intraperitoneal administration of EGCG has been well documented and proven to be an effective method of EGCG delivery (3,6,11,12). We tested a fully major histocompatibility complex (MHC) mismatched (C57BL/6 to Balb/c) (Fig. 1A), a minor mismatched (C57BL/6 to C57BL/6x129 F1) (Fig. 1B), and a xenotransplantation model (human T-cells to nonobese diabetic/severe combined immunodeficient interleukin 2 (IL-2) receptor γ chain knockout [NOD/SCID/γc KO (NSG)] (Fig. 1C). None of the recipients showed improved survival or a reduction in weight loss, suggesting that EGCG when given in the dose and schedule shown was not effective in ameliorating GvHD in any of these models. We also tested whether in vivo administration of EGCG to donor mice before harvest of donor T-cells could render the donor T-cells inactive at inducing GvHD when transplanted into allogeneic recipients (C57BL/6 to C57BL/6x129). Again, we observed no decrease of GvHD when compared to T-cells from untreated donor mice (Fig. 1D). Although we do not exclude a possibility that the doses and timing used here might not be optimal for EGCG to inhibit DNMT1 and STAT1 (our hypothesis) or to mask immunostimulatory receptors (5) and concede that refinement of EGCG treatment might result in GvHD prevention, our preliminary results do not corroborate or confirm those of Kanamune et al. (5) and suggest that the effect of EGCG on GvHD is, at best, minimal. In addition, the current clinical goal is to prevent GvHD while maintaining GvL, which, like GvHD, is also mediated by alloreactive donor T-cells. Even though it is conceivable that EGCG-treated T-cells or direct administration of EGCG to recipients might result in a reduction of GvHD, it remains to be determined whether or not it might also abrogate the beneficial GvL effect. The "immunocamouflage" of allogeneic T-cell receptors by EGCG, as hypothesized by the authors (7), is unlikely to result in differential activation of donor T-cells by tumor-associated antigens and alloantigens on GvHD target organs. Furthermore, based on the studies by Kanamune et al. (5), it is likely that EGCG-treated T-cells would possess less GvL potential since their expansion in recipients is highly limited.

In conclusion, although EGCG may be an effective inhibitor of both DNMT1 and STAT1, the treatment of EGCG after allo-HSCT does not result in prevention or reduction of GvHD in four different GvHD models, suggesting that EGCG treatment might not be an effective therapy against GvHD in the clinic.

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REFERENCES

1. Choi, J.; Ritchey, J.; Prior, J. L.; Holt, M.; Shannon, W. D.; Deych, E.; Piwnica-Worms, D. R.; DiPersio, J. F. In vivo administration of hypomethylating agents mitigate graft-versus-host disease without sacrificing graft-versus-leukemia. Blood 116(1):129–139; 2010.
2. Choi, J.; Ziga, E. D.; Ritchey, J.; Collins, L.; Prior, J. L.; Cooper, M. L.; Piwnica-Worms, D.; DiPersio, J. F. IFNgammaR signaling mediates alloreactive T-cell trafficking and GVHD. Blood 120(19):4093–4103; 2012.
3. Chyu, K. Y.; Babbidge, S. M.; Zhao, X.; Dandillaya, R.; Rietveld, A. G.; Yano, J.; Dimayuga, P.; Cercek, B.; Shah, P. K. Differential effects of green tea-derived catechin on developing versus established atherosclerosis in apolipoprotein E-null mice. Circulation 109(20):2448–2453; 2004.
4. Fang, M. Z.; Wang, Y.; Ai, N.; Hou, Z.; Sun, Y.; Lu, H.; Welsh, W.; Yang, C. S. Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. Cancer Res. 63(22):7563–7570; 2003.

5. Kanamune, J.; Iwanaga, Y.; Kina, T.; Noguchi, H.; Matsumura, K.; Uemoto, S.; Hyon, S. H. Attenuation of murine graft-versus-host disease by a tea polyphenol. Cell Transplant. 21(5):909–918; 2012.
6. Kao, Y. H.; Hiipakka, R. A.; Liao, S. Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. Endocrinology 141(3):980–987; 2000.
7. Kim, J. Y.; Kina, T.; Iwanaga, Y.; Noguchi, H.; Matsumura, K.; Hyon, S. H. Tea polyphenol inhibits allostimulation in mixed lymphocyte culture. Cell Transplant. 16(1):75–83; 2007.
8. Lyko, F.; Brown, R. DNA methyltransferase inhibitors and the development of epigenetic cancer therapies. J. Natl Cancer Inst. 97(20):1498–1506; 2005.
9. Menegazzi, M.; Tedeschi, E.; Dussin, D.; De Prati, A. C.; Cavalieri, E.; Mariotto, S.; Suzuki, H. Anti-interferon gamma action of epigallocatechin-3-gallate mediated by specific inhibition of STAT1 activation. FASEB J. 15(7): 1309–1311; 2001.
10. Rettig, M. P.; Ritchey, J. K.; Prior, J. L.; Haug, J. S.; Piwnica-Worms, D.; DiPersio, J. F. Kinetics of in vivo elimination of suicide gene-expressing T cells affects engraftment, graft-versus-host disease, and graft-versus-leukemia after allogeneic bone marrow transplantation. J. Immunol. 173(6):3620–3630; 2004.
11. Rezai-Zadeh, K.; Shytle, D.; Sun, N.; Mori, T.; Hou, H.; Jeanniton, D.; Ehrhart, J.; Townsend, K.; Zeng, J.; Morgan, D.; Hardy, J.; Town, T.; Tan, J. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. J.Neurosci. 25(38):8807–8814; 2005.
12. Siddiqui, I. A.; Asim, M.; Hafeez, B. B.; Adhami, V. M.; Tarapore, R. S.; Mukhtar, H. Green tea polyphenol EGCG blunts androgen receptor function in prostate cancer. FASEB J. 25(4):1198–1207; 2011.
13. Wong, C. P.; Nguyen, L. P.; Noh, S. K.; Bray, T. M.; Bruno, R. S.; Ho, E. Induction of regulatory T cells by green tea polyphenol EGCG. Immunol.Lett. 139(1–2):7–13; 2011.