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Brief Communication

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

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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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Figure 1. Effect of EGCG on GvHD. T-cell-depleted bone marrow (TCD BM) cells ($5 \times 10^6$) [major histocompatibility complex class II H-2b, cluster of differentiation 45.1 positive (CD45.1⁺)] along with pan T-cells (H-2b, CD45.2⁺; $5 \times 10^5$ T-cells for Balb/c and $5 \times 10^6$ for C57BL/6×129) isolated from C57BL/6 donor mice were intravenously transplanted into Balb/c recipient mice (H-2d, 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/6×129 F1 (1,200 cGy; H-2b, 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 ($3 \times 10^6$) 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 ($10^{-1}$−$1,000$ 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 ($2 \times 10^7$, CD229.2⁺; CD45.2⁺) and TCD BM ($5 \times 10^6$, CD229.2⁺; CD45.1⁺) were injected via the lateral tail vein on day 0 into lethally irradiated C57BL/6×129 recipients (1,200 cGy on day −1; H-2b, 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 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 allo-antigens 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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