Effect of Maraviroc on HIV Disease Progression-Related Biomarkers

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The potential effect of blocking the CCR5 receptor on HIV disease progression biomarkers is not well understood. We showed that an 8-day maraviroc (MVC) monotherapy clinical test (MCT) can be used in selecting patients to receive MVC-containing combined antiretroviral therapy (cART). Using this MCT model, we assessed the effect of MVC on several HIV disease progression biomarkers during the MCT (MVC-specific effect) and following short-term (12-week) cART. We compared 45 patients on MVC monotherapy with a control group of 25 patients on MVC-sparing cART. We found that MVC did not modify any biomarkers in patients that had no virological response after the MCT. MVC-specific effects in patients with virological responses included increased CD8+ T-cell activation and senescence levels, preservation of an increase in soluble CD14 (sCD14), and a decrease in D dimer levels. After 12 weeks, MVC-containing cART increased CD8+ T-cell counts and preserved CD4+ T-cell senescence levels compared with MVC-sparing cART. Moreover, there was a decrease in sCD14 levels in patients that received MVC-containing cART. In conclusion, effects compatible with CD8+ T-cell redistribution in peripheral blood were observed after MVC therapy. However, MVC was associated with a favorable profile in HIV disease progression biomarkers only in patients with a virological response. These results support a potential clinical benefit of a therapy which includes MVC in HIV-infected patients.

MATERIALS AND METHODS

Patients. Since July 2008, the MCT has been routinely used at the Infectious Diseases Service at Virgen del Rocio University Hospital for selecting candidate patients to receive MVC-containing cART (11). Briefly, the MCT consists of 8 days of MVC monotherapy exposure. The subsequent virological response is analyzed to determine whether MVC should be included in the subsequent round of cART. The MCT is considered positive if a significant viral load reduction, defined as a reduction of ≥1 log10 HIV RNA copy/ml, or an undetectable viral load (<40 HIV RNA copies/ml) was achieved on day 8 after MVC monotherapy. All of the patients were asymptomatic when the study was performed, and the inclusion criteria for the MCT have been previously defined (11). Inclusion criteria include (i) a persistently detectable viral load (>40 HIV RNA copies/ml) during the last 6 months, (ii) no highly active antiretroviral therapy (HAART) modification in the last 6 months, (iii) no HAART reintroduction in the last 6 months in patients undergoing previous supervised treatment interruption (STI), (iv) no previous treatment with coreceptor antagonists, and (v) available future therapeutic options apart from MVC. For the purpose of the present study, a subgroup of patients with available samples was included. Three groups were defined as follows. (i) The MCT-positive group (MCT+) contains patients with a positive response after the MCT (n = 30). (ii) The MCT-negative group (MCT−) includes patients without a virological response after the MCT (n = 15). MVC-containing and MVC-sparing cART, respectively, was started after the
MCT in these patients. (iii) The control group contains 25 consecutive asymptomatic treatment-naïve patients starting conventional MVC-sparing cART (n = 25). The inclusion period for these patients was the same as those for the MCT groups.

All patients were evaluated at baseline and on day 8. Furthermore, in addition to the evaluation time points in our previous study design (20), patients on follow-up were analyzed at baseline and after 12 weeks on cART. The study design is shown in Fig. 1. Patients, or legal guardians of patients under 18 years of age, provided written informed consent, and the ethical committee of the hospital approved the study.

**Laboratory tests.** Plasma HIV-1 RNA was measured in fresh samples by quantitative PCR (Cobas AmpliPrep/Cobas TaqMan HIV-1 test; Roche Molecular Systems, Basel, Switzerland) according to the manufacturer’s instructions. The lower detection limit was 40 HIV-1 RNA copies/ml. A qualitative PCR amplification was performed for plasma hepatitis C virus (HCV) RNA amplification (Cobas Amplicor; Roche Diagnostics, Barcelona, Spain), and the lower detection limit was 15 IU/ml. CD4+ T-cell counts were determined in fresh whole blood using the Epics XL-MCL flow cytometer (Beckman-Coulter, Inc., California) according to the manufacturer’s instructions.

**HIV disease progression-associated biomarkers.** Predictor biomarkers of all causes of mortality in HIV-infected patients (3, 4, 16, 24) were assayed on available frozen samples by following the manufacturer’s instructions with minor modifications. sCD14 levels, a monocyte activation biomarker, were assayed with the commercially available enzyme-linked immunosorbent assay (ELISA) kit on sera diluted 0.01% in duplicate wells (R&D Systems). D dimer levels, a thrombotic activity biomarker, were measured in an automated latex-enhanced immunosassay for the quantitative determination of D dimer in plasma samples (HemosIL D-Dimer HS 500; Instrumentation Laboratory). Levels of hsCRP, an inflammation marker, were determined with an immunoturbidimetric assay on sera using Roche automated clinical chemistry analyzers by following the manufacturer’s instructions. The sensitivity and linearity ranges were assayed in every biomarker, and they fit with the range indicated in the respective assay kit as described by the manufacturer’s instructions. There was an interassay linearity of the samples of >90% in all the assays. All the sample measures were above the detection limit of every assay.

**T-cell immunophenotyping.** CD4+ and CD8+ peripheral T-cell subsets were positively selected from available frozen peripheral blood mono-nuclear cells (PBMCs) using magnetic microbeads from magnetically activated cell sorting (MACS) cell separation reagents (Miltenyi Biotec, Germany) according to the manufacturer’s instructions. The purity obtained from the separation of both T-cell subsets was routinely >90%. CD4+ and CD8+ T cells were then stained with the conjugated monoclonal antibodies CD28-PE, CD57-FITC, HLA-DR-ECD, CD45RA-PC7, CD45-Pent, and CD38-PE (Beckman Coulter, Hialeah, FL), CD45RA-FITC, and CD45RO-PC7 (BD Biosciences, CA) and analyzed by 5-color flow cytometry performed with a Beckman Coulter Cytomics FC500 MPL flow cytometer. T-cell subsets were defined as: naïve T cells (CD45RA+ CD27+), memory T cells (CD45RO+ CD27+), effector memory T cells (CD45RO+ CD27−), and effector memory RA T cells (TemRA) (CD45RA+ CD27−). The accuracy of these phenotypes was recently reported (8). To identify activated T cells, HLA-DR+ CD38+ staining was used for CD4+ and CD8+ T cells, and CD38− staining was used for CD8+ T cells. Senescent T cells were characterized as CD57+ CD28−.

**Statistical analyses.** Continuous variables are expressed as medians with the interquartile ranges (IQR), and categorical variables are expressed as numbers and percentages. Differences between the groups were analyzed using a chi-square test to compare categorical variables, while the Mann-Whitney U test (nonpaired variables) and Wilcoxon test (paired variables) were used for continuous variables. All P values of <0.05 were considered significant. Statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS 17.0; Chicago, IL), and the graphics were generated with Prism, version 5.0 (GraphPad Software, Inc.).

## RESULTS

### Baseline characteristics of the patients

The baseline characteristics of the 70 patients included in this study are shown in Table 1. Compared with the control group, MCT+ patients exhibited higher CD4+ T-cell counts, a lower HIV load, older age, a greater length of time since HIV diagnosis, and a higher frequency of HCV coinfection. Moreover, MCT− patients had the lowest nadir and CD4+ T-cell counts, as previously described (22), and a higher proportion of these patients were in CDC stage C.

**MVC-specific effects after 8-day therapy**. The HIV viral load and T-cell levels were not modified in the MCT− group. The HIV viral load decreased significantly (P < 0.001), and the median T-cell levels increased significantly in the MCT+ group and the control group, as we previously reported (20) (Table 2). T-cell increases were similar between the MCT+ group and the control group. P value of 0.284 for CD4+ and P value of 0.251 for CD8+ T cells.

No differences in any of the studied biomarkers were observed in the MCT− group (Fig. 2A to E). CD8+ T-cell activation and senescence levels increased in the MCT+ group, while these levels remained unchanged in the control group. No changes in CD4+ T-cell activation or senescence levels were observed in either group (Fig. 2A to D). Regarding these biomarkers in the T-cell subsets, only TemRA CD4+ T-cell senescence levels decreased in the MCT+ group (P = 0.044) (Fig. 3A). Regarding inflammatory markers, sCD14 levels significantly increased in the control group while they remained stable in the MCT+ group (Fig. 2E; see also Fig. S1A in the supplemental material). Moreover, D dimer levels significantly decreased only in the MCT+ group (Fig. 2F; see also Fig. S1B in the supplemental material). Finally, hsCRP showed a trend toward decreasing only in the MCT+ group (P = 0.073) (data not shown).

**Effects after 12 weeks on cART.** Patients with follow-up at week 12 on cART were analyzed. Eleven MCT+ patients (73.3%),...
24 MCT\(^+\) patients (80.0%), and 13 patients from the control group were included in the follow-up studies (52.0%). The baseline characteristics of these subgroups were similar to those from the whole study population (see Table S1 in the supplemental material). Background antiretroviral treatments of the different groups are shown in Table S2 in the supplemental material.

All the groups demonstrated significantly decreased HIV viral load levels (Table 2), CD4\(^+\) T-cell counts significantly increased in all groups. CD8\(^+\) T-cell counts increased in the MCT\(^+\) and MCT\(^-\) groups, while in the control group, these levels remained unchanged (Table 2). The median CD4\(^+\) T-cell increases (ranges) were similar between the MCT\(^+\) group and the control group [92 (34 to 162) and 173 (68 to 210), respectively; \(P = 0.139\)].

CD4\(^+\) T-cell activation levels tended to increase, and CD4\(^+\) T-cell senescence levels significantly increased only in the groups on MVC-sparing cART (Fig. 4A and B). Moreover, only senescent TemRA CD4\(^+\) T-cell levels showed a decreasing trend in the MCT\(^+\) group, similar to the results of the 8-day treatment (\(P = 0.061\)) (Fig. 3B). CD8\(^+\) T-cell activation levels tended to decrease in the MCT\(^+\) group and significantly decreased in the control group (Fig. 4C). When we considered the activation phenotype to be CD38\(^+\) HLA-DR\(^+\), we found similar results with even more significant decreases (\(P = 0.008\) for MCT\(^+\) patients and \(P = 0.048\) for control group patients). Additionally, increased CD8\(^+\) T-cell senescence levels were found only in the control group, and these differences remained significant when the outlier value at week 12 was eliminated from the control group (Fig. 4D).

Interestingly, sCD14 levels decreased in the MCT\(^+\) patients, and there was a decreasing trend in the MCT\(^-\) group. However, there were no changes in the control group (Fig. 4E; see also Fig. S2A in the supplemental material). The MCT\(^+\) and control groups showed a significant decrease in D dimer levels (Fig. 4F; see also Fig. S2B in the supplemental material). The short-term cART effects on hsCRP levels were similar in all groups, showing no significant changes (data not shown).

**DISCUSSION**

Eight days of MVC monotherapy allowed us to determine the specific effects of blocking R5 on several HIV disease progression-related biomarkers. First, MVC did not modify any biomarkers in MCT\(^+\) patients. In contrast, an increase in both CD8\(^+\) T-cell activation and senescence levels and a favorable profile of D dimer and sCD14 levels were observed in MCT\(^+\) patients. In addition, 12 weeks of MVC-containing cART significantly increased the absolute CD8\(^+\) T-cell counts and maintained lower CD4\(^+\) T-cell senescence levels compared with the values for the cART group. Similarly, MVC-containing cART decreased sCD14 levels compared with those of an MVC-sparing cART.

We have previously shown that MVC-specific effects on T-cell changes are dependent on the drug’s antiretroviral activity (20). We analyzed this issue in depth with respect to HIV disease progression biomarkers. In accordance with our previous results, the

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**TABLE 1 Baseline characteristics of the patients**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median value (IQR) in each group(^a)</th>
<th>(P) value for(^b):</th>
<th>MCT(^+) vs control</th>
<th>MCT(^-) vs control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>40 (37–45)</td>
<td>44 (40–48)</td>
<td>37 (28–47)</td>
<td>NS</td>
</tr>
<tr>
<td>No. (%) male</td>
<td>13 (86.7)</td>
<td>22 (73.3)</td>
<td>19 (76.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Time from HIV diagnosis (wk)</td>
<td>858 (769–1,014)</td>
<td>950 (596–1,061)</td>
<td>30 (9–57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. (%) in CDC clinical stage C</td>
<td>74 (46.7)</td>
<td>6 (20.0)</td>
<td>3 (12.0)</td>
<td>0.024</td>
</tr>
<tr>
<td>CD4(^+) T cells (cells/mm(^3))</td>
<td>77 (17–164)</td>
<td>287 (221–456)</td>
<td>224 (122–320)</td>
<td>0.005</td>
</tr>
<tr>
<td>Nadir CD4(^+) T cells (cells/mm(^3))</td>
<td>33 (4–77)</td>
<td>190 (112–282)</td>
<td>190 (103–348)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD8(^+) T cells (cells/mm(^3))</td>
<td>437 (372–1,236)</td>
<td>826 (577–1,037)</td>
<td>617 (458–938)</td>
<td>NS</td>
</tr>
<tr>
<td>HIV load (log(_{10}) RNA copies/ml)</td>
<td>4.9 (3.7–5.3)</td>
<td>4.2 (2.9–4.9)</td>
<td>4.8 (4.3–5.2)</td>
<td>NS</td>
</tr>
<tr>
<td>No. (%) HCV PCR(^+)</td>
<td>6 (40.0)</td>
<td>13 (43.3)</td>
<td>3 (12.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^a\) Data are expressed as the median (interquartile range) of all participants unless otherwise specified.

\(^b\) NS, not significant.

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**TABLE 2 Absolute T-cell and HIV viral load changes after 8-day therapy and 12 weeks on cART**

<table>
<thead>
<tr>
<th>Measured change</th>
<th>Therapy</th>
<th>Median (IQR) change and (P) value in each group(^a)</th>
<th>(P)</th>
<th>MCT(^+)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MCT(^+)</td>
<td>MCT(^-)</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>CD4(^+) T cells (cells/mm(^3))</td>
<td>8-day therapy</td>
<td>2 (–5 to 22)</td>
<td>0.798</td>
<td>67 (30 to 117)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>12-wk cART</td>
<td>53 (16 to 102)</td>
<td>0.029</td>
<td>92 (34 to 162)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD8(^+) T cells (cells/mm(^3))</td>
<td>8-day therapy</td>
<td>42 (–108 to 294)</td>
<td>0.426</td>
<td>264 (76 to 542)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>12-wk cART</td>
<td>135 (53 to 357)</td>
<td>0.014</td>
<td>91 (12 to 386)</td>
<td>0.004</td>
</tr>
<tr>
<td>HIV load (log(_{10}) RNA copies/ml)</td>
<td>8-day therapy</td>
<td>0.05 (–0.17 to 0.23)</td>
<td>0.496</td>
<td>–1.23 (–1.66 to –1.03)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>12-wk cART</td>
<td>–3.30 (–3.38 to –2.32)</td>
<td>0.003</td>
<td>–2.44 (–3.01 to –1.47)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^a\) The Wilcoxon test was used to study T-cell and HIV load levels between day 0 and day 8 and between day 0 and week 12 along the follow-up in the different groups. \(P\) values of <0.05 were considered statistically significant and are indicated in bold.
first important finding of this study is that MVC had no effect on any of the measured parameters in the patients who had no virological response. This finding supports the fact that an antiretroviral effect is necessary to observe the specific immune effects of MVC in relation to these markers. Thus, MVC will not have any immunomodulatory effect on patients who are insensitive to the drug, at least on the parameters studied herein, a finding which is in contrast to what has been previously suggested (23).

Surprisingly, the MVC-specific effect after 8 days was an increase in CD8⁺ T-cell activation and senescence levels. The fact

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**FIG 2** MVC-specific effects after eight-day therapy. The results are expressed as absolute T-cell numbers (cells/mm³) at day 0 (D0) and day 8 (D8) for every study group. (A and B) CD4⁺ T-cell activation (A) and senescence (B) levels. (C and D) CD8⁺ T-cell activation (C) and senescence (D) levels. (E and F) sCD14 and D dimer levels (E and F, respectively). Horizontal gray bars represent the median values for each data point. Differences along the follow-up were studied using the Wilcoxon test; significant values are depicted with an asterisk. Data from the MCT-negative group (MCT⁻) are represented as triangles, data from the MCT-positive group (MCT⁺) are shown as circles, and data from the control group are shown as squares. The numbers of analyzed patients in the MCT⁻, MCT⁺, and control groups were 12, 28, and 24 (A), 12, 29, and 24 (B), 15, 29, and 23 (C), 14, 27, and 20 (D), 11, 27, and 25 (E), and 9, 19, and 23 (F), respectively.
that these effects ceased at the onset of the cART after the MCT, even with MVC-containing cART, may suggest that blocking CCR5 causes early lymphocyte redistribution from the lymph nodes to blood (5, 21) rather than a proactivation effect of MVC. In fact, T-cell redistribution from lymph to blood has been shown to occur early after HAART, although this effect has not been studied at such a short term as in this study (5). In addition, MVC showed a favorable specific effect on soluble markers associated with HIV disease progression, demonstrated by significant decreases in D dimer together with sCD14 stabilization and a trend toward decreasing hscRP levels compared with the control group, although levels at day 8 were similar for the two therapies.

The results presented here shed light on the potential clinical beneficial effects of MVC-containing cART. In fact, when we analyzed the short-term cART effect, we observed how MVC differentially diminished sCD14 levels. This decrease in a marker of innate immune activation may have important clinical implications because high sCD14 levels have been independently associated with mortality in HIV-infected patients (24). The decrease of D dimer levels which have been associated with lower cardiovascular risk in HIV-infected patients (4) could also be clinically relevant. However, these results contradict the increase in sCD14 levels observed after MVC intensification in a recent study (14). In fact, the higher levels found in that study were statistically significant after 24 to 48 weeks, but not at 12 weeks, on cART, and the previous study included a very different set of patients (under an icant after 24 to 48 weeks, but not at 12 weeks, on cART, and the fact, the higher levels found in that study were statistically signif-

FIG 3 Absolute TemRA senescent CD4+ T-cell levels after 8-day MVC monotherapy and after 12 weeks on cART. The figures show senescent TemRA CD4+ T-cell numbers (cells/mm3) at day 0 and day 8 for every study group in panel A and senescent TemRA CD4+ T-cell numbers (cells/mm3) at day 0 and week 12 (W12) for every study group in panel B. Horizontal gray bars represent the median values for each data point. Differences during the follow-up were studied using the Wilcoxon test; significant values are depicted with an asterisk. Data from the MCT-negative group (MCT-) are represented as triangles, data from the MCT-positive group (MCT+) are shown as circles, and data from the control group are shown as squares. The numbers of analyzed patients in the MCT-, MCT+, and control groups were 6, 22, and 19 (A) and 5, 19, and 9 (B), respectively.

This work has some limitations, including the fact that groups that underwent MCT were pretreated and were older, had a greater length of time since HIV diagnosis, and had a higher proportion of HCV coinfection than the control group, which was composed of naïve patients. This different composition of the groups was mandatory when this study was designed and performed because MVC prescription in Europe was indicated only in pretreated patients at that time. These differences may negatively impact the parameters measured in the MCT+ group. However, we observed similar CD4+ T-cell changes after therapy and, in general, a more favorable immunological profile in these patients than in the control group. Therefore, we would expect even greater differences favoring the MCT+ group if the variables mentioned above were controlled for among the patients. We do not know if a longer time on MVC monotherapy would have favored additional MVC-specific effects. This final limitation may also be considered a strength of the study, reflecting the unique design of the MCT, which led us to analyze the MVC-specific effect.
In conclusion, MVC-specific effects induced a favorable profile in D-dimer and sCD14 levels, a result which is opposite of the effects observed in T-cell activation. The reversible nature of the latter effect after cART suggests that this phenomenon is compatible with a redistribution of T cells from the lymph nodes in the short term. MVC-containing cART seems to induce a more favorable profile in HIV disease progression-related markers, such as sCD14, CD4⁺ T-cell activation, and T-cell senescence. These ob-

FIG 4 Effect of MVC-containing or MVC-sparing cART after 12 weeks. The results are expressed as absolute T-cell numbers (cells/mm³) at day 0 and week 12 for every study group. (A and B) CD4⁺ T-cell activation (A) and senescence (B) levels. (C and D) CD8⁺ T-cell activation (C) and senescence (D) levels. (E and F) sCD14 and D-dimer levels (E and F, respectively). Horizontal gray bars represent the median values for each data point. Differences along the follow-up were studied using the Wilcoxon test; significant values are depicted with an asterisk. Data from the MCT-negative group (MCT⁻) are represented as triangles, data from the MCT-positive group (MCT⁺) are shown as circles, and data from the control group are shown as squares. The numbers of analyzed patients in the MCT⁻, MCT⁺, and control groups were 8, 22, and 13 (A), 10, 23, and 13 (B), 10, 22, and 13 (C), 9, 22, and 9 (D), 9, 22, and 13 (E), and 8, 15, and 13 (F), respectively.
servations are particularly important in the current cART era, in which new immunotherapeutic approaches to decrease HIV disease progression markers, beyond the antiretroviral effect, are needed.

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