Metabolism of 15-Hydroxy-5,8,11,13-eicosatetraenoic Acid by MOLT-4 Cells and Blood T-lymphocytes*

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Christos Hadjiagapiou, Jeffrey B. Travers, Richard H. Fertel, and Howard Sprecher

From the Departments of Physiology and Pharmacology, Ohio State University, Columbus, Ohio 43210

MOLT-4 lymphocytes metabolize 15-hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE) via \( \beta \)-oxidation with retention of the hydroxyl group at the \( \omega \)-carbon atom. 15-HETE oxidation is accompanied by the time-dependent accumulation of both \( \beta \)-hydroxy acids and metabolites produced by repetitive cycles of the \( \beta \)-oxidation spiral. Detection of 7-hydroxy-5,8,11-octodecenoic acid shows that these cells continue to \( \beta \)-oxidize the substrate when the conjugated diene is allylic to a hydroxyl group. When 15-HETE was the substrate, it was also possible to detect 12-hydroxy-5,8,10-heptadecatrien-1-al and 3,15-dihydroxy-8,11,13-eicosatrienoic acid. The former product may be produced by \( \alpha \)-oxidation of 13-hydroxy-8,9,11-octadecatrienoic acid followed by its deacylation. Detection of a 20-carbon metabolite, lacking a double bond at position 5, suggests that an intermediate of \( \beta \)-oxidation was used as a substrate for chain elongation. When 13-hydroxy-6,9,11-octadecatrienoic acid was used as a substrate, it was indeed possible to detect 3,15-dihydroxy-8,11,13-eicosatrienoic acid as well as 15-hydroxy-8,11,13-eicosatrienoic acid. In addition, 13-hydroxy-6,9,11-octadecatrienoic acid was a precursor for the synthesis of both 14-hydroxy-7,10,12-nonadecatrien-1-al and 1,14-dihydroxy-7,10,12-nonadecatriene. These studies with MOLT-4 cells as well as with T-lymphocytes isolated from blood show that products of the 15-lipoxygenase pathway are metabolized with the accumulation of a variety of compounds. Since 15-HETE has been implicated as a modulator of T-cell function, these findings raise the possibility that the newly described metabolites may be involved in regulating lymphocyte function.

T-lymphocytes isolated from human blood probably do not metabolize arachidonic acid via the cyclooxygenase or lipoxigenase pathway (1). However, several lymphocyte cell lines that are responsive to interleukins-1 and -2 synthesize immunoreactive 5-hydroxy-6,8,11,14-eicosatetraenoic acid and 15-HETE upon stimulation with phorbol esters, the calcium ionophore A23187, or interleukin-1 or -2 (2). 15-HETE inhibits lectin-stimulated T-lymphocyte proliferation (3, 4). In addition, 15-HETE inhibits the stimulation of lymphocyte guanylate cyclase that occurs rapidly after addition of mito-

DISCUSSION

The pathway for the \( \beta \)-oxidation of unsaturated fatty acids has recently been reviewed by Schultz and Kunau (27). According to this pathway, a fatty acid with its first double bond at position 4 is converted by an acyl dehydrogenase into a \( \beta \)-oxidation product which is then isomerized to the \( \beta \)-oxidation product of the \( \Delta^5 \)-isomerase. This latter enzyme is also required for \( \beta \)-oxidation of fatty acids which contain double bonds in the \( \omega \)-carbon atom. Generally, long chain intermediates do not accumulate during fatty acid oxidation. An exception is the perfused ischemic rat heart, where 12-, 14-, and 16-carbon \( \beta \)-hydroxy acids are detected when palmitic acid is the substrate (28). Fig. 1 shows that it is possible to detect 10 metabolites when MOLT-4 cells are incubated with 15-HETE. Fig. 9 depicts a flow chart showing a possible metabolic pathway for the synthesis of these compounds. The metabolism of 15-HETE results in the accumulation of seven compounds which are expected products of 15-HETE. \( \beta \)-oxidation.
Metabolism of 15-HETE

The intermediates which accumulate are β-hydroxy acids and metabolites that would be expected by a complete revolution of the β-oxidation cycle. We did not detect any β-keto acids or acids with a 2-trans double bond. These cells appear to contain all the enzymes expected for unsaturated fatty acid oxidation. The conversion of 13-ΟΗ-3,6,9,11-18:4 to 3,13-diΟΗ-6,9,11-18:3 implies that lymphocytes have the Δ2-cis-Δ-trans-enoyl-CoA isomerase. Hydration of the resulting 13-ΟΗ-2-trans-6,9,11-18:4 would give 3,13-diΟΗ-6,9,11-18:3.

The principal metabolite that accumulated was 11-ΟΗ-4,7,9-16:3. This also is the metabolite that accumulates when 15-HETE is incubated with endothelial cells (29). Likewise, 8-ΟΗ-6,9,10-16:3 accumulates as the major metabolite when 12-HETE is incubated with vascular smooth muscle cells (30). The significance of these findings is not readily apparent, but it does suggest that all these cells may have a low level of the acyl-CoA dehydrogenase that introduces a double bond at the 2-trans position to form a conjugated diene.

The metabolism of 9-ΟΗ-5,7-14:2 to 7-ΟΗ-12:1 presumably would proceed according to the following pathway: 9-ΟΗ-5,7-14:2 → 9-ΟΗ-2-trans-5,7-14:3 → 9,12-diΟΗ-5,7-14:2 → 3-keto-9-ΟΗ-5,7-14:2 → 7-ΟΗ-3,5-12:2. We did not detect 7-ΟΗ-3,5-12:2 in these studies; but rather, we found 7-ΟΗ-5-12:1. These findings suggest that the enoyl-CoA isomerase acts on the conjugated diene, 7-ΟΗ-3,5-12:2, to give 7-ΟΗ-2-trans-5,7-12:2.

A compound with a 2-trans double bond may serve as a substrate for further oxidation by hydration; or alternatively, the 2-trans bond may be reduced by an intramitochondrial nucleotide-dependent 2-trans-enoyl-CoA reductase (31). That we were able to detect 7-ΟΗ-12:1 suggests that this latter pathway is preferred in these cells. However, the failure to detect β-oxidation metabolites from 7-ΟΗ-5-12:1 does not preclude that they were not formed. If small amounts did accumulate, they might not be extracted from an aqueous medium by ethyl acetate.

When MOLT-4 cells were incubated with 15-HETE, it was possible to isolate 13-ΟΗ-6,9,11-18:3. This compound may be formed via two pathways. The double bond at position 3 in 13-ΟΗ-3,6,9,11-18:4 must be isomerized to the 2-trans position prior to hydration to yield 3,13-diΟΗ-6,9,11-18:3. Reduction of the 2-trans double bond would, however, yield 13-ΟΗ-6,9,11-18:3. Alternatively, 11-ΟΗ-4,7,9-16:3 may serve as a substrate for either mitochondrial or microsomal chain elongation to give 13-ΟΗ-6,9,11-18:3.

The mass spectra of the native and hydrogenated trimethylsilyl ethers of the compound labeled 12-ΟΗ-5,8,10-17:3-1-al in Fig. 9 were inconclusive in establishing its structure since it did not allow us to distinguish between an aldehyde and the isomeric methyl ketone. A methyl ketone is an expected product since β-keto acids undergo spontaneous decarboxylation during extraction (32). 3-Keto-13-ΟΗ-6,9,11-18:3 is the presumed intermediate in the conversion of 3,13-diΟΗ-6,9,11-18:3 to 11-ΟΗ-4,7,9-16:3. However, when the double bonds and the carboxyl group were reduced, the resulting spectrum of the trimethylsilyl ether is consistent with the structure in Fig. 9. In Fig. 9, we suggest that 13-ΟΗ-6,9,11-18:3 is the precursor of 12-ΟΗ-5,8,10-17:3-1-al. The pathway implies α-oxidation followed by decarboxylation, but it has not yet been verified. It is, however, at least consistent with the finding that the 17-carbon aldehyde is formed when MOLT-4 cells are incubated with 13-ΟΗ-6,9,11-18:3 (Fig. 10).

The identification of 3,13-diΟΗ-8,11,13-20:3 as a product of 15-HETE metabolism suggests that a catalytic intermediate serves as a substrate for chain elongation. The flow chart in Fig. 10 depicts a possible metabolic pathway for the compounds that were detected when 13-ΟΗ-6,9,11-18:3 was incubated with MOLT-4 cells. In addition to 3,15-diΟΗ-8,11,13-20:3, the fully catalyzed product, 15-ΟΗ-8,11,13-20:3, was produced in small amounts. In addition, a 19-carbon aldehyde and 1,4 diΟΗ-7,10,12-19:3 were produced. These findings suggest that the aldehyde is reduced to an alcohol. We synthesized 7,10,13-trinadecatrien-1-al and attempted to hydroxylate it at the α-carbon by reaction with soybean lipoxigenase. However, we were only able to recover starting material, so we could not evaluate whether the 19-carbon aldehyde was a substrate for nucleotide-dependent reduction. This reaction, like the conversion of 15-ΟΗ-8,11,13-20:3 to 14-ΟΗ-7,10,12-19:3-1-al, has not yet been verified.

Previous studies have shown that 15-HETE is metabolized via a number of oxidative pathways without altering the chain length of the substrate. For example, 15-HETE is metabolized in neutrophils by the 5-lipoxygenase into lipoxins A and B (33). A number of dihydroxy acids are produced when 15-HETE is incubated with purified porcine leukocyte 12-lipoxygenase (reviewed in Ref. 34). 15-HETE is oxidized by 15-hydroxyprostaglandin dehydrogenase to 15-keto-5,8,11,13-eicosatetraenoic acid (35). Shen et al. (29) have recently shown that endothelial cells metabolize 15-HETE via a second general pathway which involves β-oxidation with the accumulation of 11-hydroxy-4,7,9-hexadecatrienoic acid.

Bailey et al. (3) reported that ~30 μM 15-HETE inhibited lymphocyte mitogenesis by about 50%. This concentration is similar to that used in our studies to define 15-HETE metabolites.
Metabolism in MOLT-4 cells and blood T-lymphocytes. Although the time-dependent metabolism of 15-HETE by blood T-lymphocytes was not defined, we did detect seven metabolites after a 24-h incubation. The major metabolite was 11-OH-4,7,9,16:3. In addition, we identified 12-hydroxy-5,8,10-heptadecatrien-1-al. These findings show that blood T-lymphocytes metabolize 15-HETE via a similar pathway as do transformed cells. Although 15-HETE is produced by a number of cells, its localized concentration in vivo is unknown. It thus remains to be determined whether these catabolic products are made in vivo and, if they are, whether they modulate lymphocyte function.

REFERENCES

Mass spectrometry was carried out with a Hewlett-Packard model 5972A mass selective detector and a 5975A gas chromatograph. The capillary column (Altech Associates, model 600-2) was 20 ft. \( \times \) 0.5 mm i.d. \( \times \) 0.008 in. w.f. The temperature in the oven was 200°C, and the inlet and interface temperatures were 250°C. The carrier gas was helium at a flow rate of 0.1 ml/min. The ionization energy was 70 eV, and the accelerating voltage was 2500 V. The selected ion monitoring was completed on \( m/z \) 159, 201, and 229. The abundance of the ions at these m/z values was monitored as a function of time with the aid of a 32-bit computer. The results were analyzed using a Hewlett-Packard ChemStation. The retention times were measured using a HP 3392A integrator.

**Table 1**

<table>
<thead>
<tr>
<th>Method</th>
<th>Radioactivity (cpm)</th>
<th>Retention Time (min)</th>
<th>Specific Activity (cpm/mg)</th>
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</thead>
<tbody>
<tr>
<td>Reversed phase HPLC</td>
<td>159</td>
<td>0.05</td>
<td>900</td>
</tr>
<tr>
<td>Reversed phase HPLC</td>
<td>201</td>
<td>0.1</td>
<td>2000</td>
</tr>
<tr>
<td>Reversed phase HPLC</td>
<td>229</td>
<td>0.2</td>
<td>3000</td>
</tr>
</tbody>
</table>

**Figure 1**

Reverse phase HPLC chromatography of the products produced when NAD-4 cells were incubated with [3H]-15-HETE for 24 h.

**Figure 2**

Mass spectrum of the methyl ester of 15-HETE.

**Figure 3**

Mass spectrum of the methyl ester of 15-HETE after hydrolysis.

**Figure 4**

Mass spectrum of the methyl ester of 15-HETE after hydrolysis.

Although compound 2 eluted immediately after compound 1 on reverse phase HPLC, its EI mass spectrum was identical to that of compound 1. This finding, coupled with the observation that this compound was not produced upon prolonged incubation with 15-HETE, suggests that it is a short chain polar metabolite. The EI mass spectrum of compound 2 showed m/z 159, 201, and 229 as the major ions. The observed retention time of compound 2 was 0.15 min. The mass spectrum of compound 2 is interpreted as a 1,3,5-trihydroxy-3,5,7-trimethylheptane (2). The mass spectrum of the methyl ester of 15-HETE shows a peak at m/z 159, corresponding to the molecular ion. The mass spectrum of the methyl ester of compound 2 is shown in Figure 2. The mass spectrum of the methyl ester of 15-HETE after hydrolysis is shown in Figure 3. The mass spectrum of the methyl ester of compound 2 after hydrolysis is shown in Figure 4.

**Figure 5**

Mass spectrum of the methyl ester of 15-HETE after hydrolysis.
Metabolism of 15-HETE

The properties of metabolite 14 also were not altered by reaction with diazomethane. The mass spectrum of the IRS ether (Eu-70.9) had ions at m/z = 324 (M+7), 295 (M-7), 367.75, 376 (M-10), 375 (M-11), 252 (M-35), 329 (M-60), 327 (M-60), 326 (M-59), 291 (M-85), 289 (M-87), 288 (M-87), 287 (M-87), 286 (M-87), 285 (M-87), 284 (M-87), 283 (M-87), 282 (M-87), 281 (M-87), 280 (M-87), 279 (M-87), 278 (M-87), 277 (M-87), 276 (M-87), 275 (M-87), 274 (M-87), 273 (M-87), 272 (M-87), 271 (M-87), 270 (M-87), 269 (M-87), 268 (M-87), 267 (M-87), 266 (M-87), 265 (M-87), 264 (M-87), 263 (M-87), 262 (M-87), 261 (M-87), 260 (M-87), 259 (M-87), 258 (M-87), 257 (M-87), 256 (M-87), 255 (M-87), 254 (M-87), 253 (M-87), 252 (M-87), 251 (M-87), 250 (M-87), 249 (M-87), 248 (M-87), 247 (M-87), 246 (M-87), 245 (M-87), 244 (M-87), 243 (M-87), 242 (M-87), 241 (M-87), 240 (M-87), 239 (M-87), 238 (M-87), 237 (M-87), 236 (M-87), 235 (M-87), 234 (M-87), 233 (M-87), 232 (M-87), 231 (M-87), 230 (M-87), 229 (M-87), 228 (M-87), 227 (M-87), 226 (M-87), 225 (M-87), 224 (M-87), 223 (M-87), 222 (M-87), 221 (M-87), 220 (M-87), 219 (M-87), 218 (M-87), 217 (M-87), 216 (M-87), 215 (M-87), 214 (M-87), 213 (M-87), 212 (M-87), 211 (M-87), 210 (M-87), 209 (M-87), 208 (M-87), 207 (M-87), 206 (M-87), 205 (M-87), 204 (M-87), 203 (M-87), 202 (M-87), 201 (M-87), 200 (M-87), 199 (M-87), 198 (M-87), 197 (M-87), 196 (M-87), 195 (M-87), 194 (M-87), 193 (M-87), 192 (M-87), 191 (M-87), 190 (M-87), 189 (M-87), 188 (M-87), 187 (M-87), 186 (M-87), 185 (M-87), 184 (M-87), 183 (M-87), 182 (M-87), 181 (M-87), 180 (M-87), 179 (M-87), 178 (M-87), 177 (M-87), 176 (M-87), 175 (M-87), 174 (M-87), 173 (M-87), 172 (M-87), 171 (M-87), 170 (M-87), 169 (M-87), 168 (M-87), 167 (M-87), 166 (M-87), 165 (M-87), 164 (M-87), 163 (M-87), 162 (M-87), 161 (M-87), 160 (M-87), 159 (M-87), 158 (M-87), 157 (M-87), 156 (M-87), 155 (M-87), 154 (M-87), 153 (M-87), 152 (M-87), 151 (M-87), 150 (M-87), 149 (M-87), 148 (M-87), 147 (M-87), 146 (M-87), 145 (M-87), 144 (M-87), 143 (M-87), 142 (M-87), 141 (M-87), 140 (M-87), 139 (M-87), 138 (M-87), 137 (M-87), 136 (M-87), 135 (M-87), 134 (M-87), 133 (M-87), 132 (M-87), 131 (M-87), 130 (M-87), 129 (M-87), 128 (M-87), 127 (M-87), 126 (M-87), 125 (M-87), 124 (M-87), 123 (M-87), 122 (M-87), 121 (M-87), 120 (M-87), 119 (M-87), 118 (M-87), 117 (M-87), 116 (M-87), 115 (M-87), 114 (M-87), 113 (M-87), 112 (M-87), 111 (M-87), 110 (M-87), 109 (M-87), 108 (M-87), 107 (M-87), 106 (M-87), 105 (M-87), 104 (M-87), 103 (M-87), 102 (M-87), 101 (M-87), 100 (M-87), 99 (M-87), 98 (M-87), 97 (M-87), 96 (M-87), 95 (M-87), 94 (M-87), 93 (M-87), 92 (M-87), 91 (M-87), 90 (M-87), 89 (M-87), 88 (M-87), 87 (M-87), 86 (M-87), 85 (M-87), 84 (M-87), 83 (M-87), 82 (M-87), 81 (M-87), 80 (M-87), 79 (M-87), 78 (M-87), 77 (M-87), 76 (M-87), 75 (M-87).
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