Urate Production by Human Heart

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Urate Production by Human Heart. Journal of Molecular and Cellular Cardiology (1989) 21, 691-695. Xanthine oxidoreductase has been demonstrated in the heart of various species. However, its presence in human heart is still debated. In the literature, high to undetectable levels have been reported. We studied the arterial-venous mate difference across the heart of patients undergoing both routine cardiac catheterization and percutaneous transluminal coronary angioplasty. Urate is the end product of the reaction catalysed by xanthine oxidoreductase. In 10 patients, studied before angioplasty, the plasma mate level in the great cardiac vein exceeded the arterial one by 26 ± 10 nmol/ml (P = 0.028). In a further 13 patients, urate production was maximal immediately after the last of four consecutive occlusions (23 ± 8 nmol/ml, P = 0.018) and concomitant with increased coronary sinus hypoxanthine levels. We conclude that xanthine oxidoreductase is probably present in the heart of patients, suffering from ischemic heart disease, and responsible for the increase in urate production during transient myocardial ischemia.

KEY WORDS: Xanthine oxidase; Uric acid; Myocardium; Ischemia; Human; Coronary angioplasty.

Introduction

Xanthine oxidoreductase activity has been demonstrated in the myocardium of a number of species (see Schoutsen and De Jong, 1987). Limited data are available on the enzyme in human heart. Autopsy material indicates high xanthine oxidase activity (Krenitsky et al., 1974; Wajner and Harkness, 1988). Histological techniques have shown large amounts of the enzyme in human heart endothelium (Jarasch et al., 1986). On the other hand, several authors have reported very low to undetectable xanthine oxidoreductase activity in human heart (Ramboer, 1969; Eddy et al., 1987; Muxfeldt and Schaper, 1987). Preliminary observations assessing cardiac urate production in patients during pacing stress test at the University of Alabama (Nelson et al., 1977) and in patients during coronary angiography in the National Institute of Cardiology, Warsaw (Czarnecki, 1988) have suggested that the human heart may be capable of urate production. We present evidence which shows that the human heart can produce significant amounts of urate. This observation suggests that a cardiac xanthine oxidoreductase is active in patients with ischemic heart disease.

Methods

Patients

Two studies were performed in patients, catheterized for percutaneous transluminal coronary angioplasty (PTCA). In 10 patients the urate concentrations of arterial and great cardiac vein plasma, obtained before PTCA,
TABLE 1. Clinical characteristics of the study groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year), average range</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>7/3</td>
<td>10/3</td>
</tr>
<tr>
<td>CCS grade before PTCA</td>
<td>III to IV</td>
<td>II to IV</td>
</tr>
<tr>
<td>Average severity of stenosis (%) before PTCA</td>
<td>78</td>
<td>79</td>
</tr>
<tr>
<td>after PTCA</td>
<td>44</td>
<td>37</td>
</tr>
</tbody>
</table>

CCS = Canadian Cardiovascular Society.

were assayed retrospectively (Group 1). Subsequently, in a prospective study (Group 2, 13 patients), urate and hypoxanthine concentrations were measured in arterial and coronary sinus plasma before, during and after angioplasty. In both studies, arterial blood was taken from the femoral artery. Great cardiac vein blood was sampled via the distal opening of a Webster flow catheter (Group 1) and coronary sinus blood via a diagnostic catheter (Group 2). All patients had a proximal stenosis < 1 cm from the origin of the left anterior descending artery and no collateral filling to the region supplied by the artery, seen at angiography. Amipaque or Isovue contrast agents (Nyegaard, Oslo, Norway) were used for angiography. In all patients, vasoactive substances, except short-lasting nitrates, were discontinued at least 12 h before the study. The clinical characteristics are listed in Table 1.

Assays

To prepare plasma, blood was mixed in a heparinized tube with an equal volume of ice-cold 154 mM NaCl, containing 20 μM dipyridamole (Boehringer, Ingelheim, GFR) and 10 μM erytho-9-(2-hydroxy-3-nonyl)adenine (Wellcome, London, UK). These drugs were used to inhibit adenosine uptake and breakdown (Ontyd and Schrader, 1984; Edlund et al., 1985). The plasma was kept at −80°C. Deproteinization was carried out with an equal volume of 8% HClO₄ (w/v) and the supernatant fraction neutralized with 2 M KOH/1 M K₂CO₃. HPLC-determination of urate and hypoxanthine concentrations in the plasma extract were performed on a μBondapak C₁₈ column. A 100 μl sample was eluted with a mixture of CH₃OH (100 ml) and KH₂PO₄ (10 g/l, 1000 ml), pH 5-7, at a flow
rate of 0.6 ml/min. The column was equipped with a LC-18 guard-column (Supelco, Bellefonte, PA). The Waters-HPLC equipment consisted of: WISP 710B cooled autosampler, Model 6000A pump, Model 490 multi wavelength detector, and Model 840 computer. Peaks were identified by retention times, internal standards and enzyme shifts. The optimal wavelengths for urate and hypoxanthine detection proved to be 295 and 254 nm as at these levels adsorption was maximal and disturbance by other materials minimal (Fig. 1). Sample preparation and assay were based on earlier work (Harmsen et al., 1981). In 27 arterial and venous plasma samples of Group 2, urate was also assayed spectrophotometrically with uricase according to Scheibe et al. (1974). Enzyme was provided by Boehringer (Mannheim, GFR). Comparison of the data obtained with both methods showed that they correlated closely.

Data presented were analysed with Student's t-test for paired variates, or, where appropriate with two-way analysis of variance. A P value of <0.05 was considered as significant. The correlation test was done according to Bland and Altman (1986).

**Results**

In the preliminary studies, mentioned in the Introduction, hearts produced urate. In the American study, the arterial and venous blood urate levels were 59 ± 20 and 120 ± 23 nmol/ml, resp. (n = 7, P = 0.003). In the Polish study, these values were 96 ± 15 and 145 ± 25 nmol/ml resp. (n = 6, P = 0.028).

In Group 1, all patients had an isolated proximal left anterior descending artery stenosis and angina pectoris. In this group the arterial urate concentration was significantly lower than the coronary venous one (216 ± 17 and 242 ± 17 nmol/ml); a difference of 26 nmol/ml (P = 0.028). In seven out of 10 patients, the heart produced urate.

In a comparable patient population (Group 2, see Table 2), plasma urate concentrations were similar to those of Group 1, but the arterio-venous difference before coronary angioplasty was relatively small. Consequently we were unable to demonstrate significant urate production before coronary angioplasty. Analysis of data showed a significant increase in urate production during balloon inflations (F = 2.85; P < 0.05). *After* the third and fourth inflations, venous urate levels were significantly higher than arterial ones (Fig. 2). They differed 14 nmol/ml (P = 0.009) and 23 nmol/ml (P = 0.018), resp. Even after 15 min of recovery, urate production was still significant. The difference was 7 nmol/ml (P = 0.033).

In the latter study we also measured the arterial and coronary sinus hypoxanthine levels with HPLC. The arterial hypoxanthine

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**TABLE 2. Arterial and venous urate levels before, immediately after four dilations, and during recovery**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Before PTCA</th>
<th>After 1st dilation</th>
<th>After 2nd dilation</th>
<th>After 3rd dilation</th>
<th>After 4th dilation</th>
<th>Recovery</th>
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<td>ART CS</td>
<td>ART CS</td>
<td>ART CS</td>
<td>ART CS</td>
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<tr>
<td>1</td>
<td>217 211</td>
<td>214 217</td>
<td>211 217</td>
<td>218 216</td>
<td>212 217</td>
<td>215 218</td>
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<tr>
<td>2</td>
<td>236 216</td>
<td>224 184</td>
<td>216 195</td>
<td>213 207</td>
<td>213 200</td>
<td>202 196</td>
</tr>
<tr>
<td>3</td>
<td>224 229</td>
<td>223 220</td>
<td>218 215</td>
<td>218 219</td>
<td>217 217</td>
<td>215 211</td>
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<tr>
<td>4</td>
<td>279 291</td>
<td>280 290</td>
<td>279 281</td>
<td>236 292</td>
<td>245 284</td>
<td>262 262</td>
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<td>5</td>
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<td>231 266</td>
<td>239 257</td>
<td>244 260</td>
<td>200 266</td>
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<td>6</td>
<td>216 224</td>
<td>219 221</td>
<td>208 216</td>
<td>211 218</td>
<td>209 216</td>
<td>202 206</td>
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<td>7</td>
<td>252 261</td>
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<td>230 242</td>
<td>218 214</td>
<td>201 207</td>
<td>197 209</td>
<td>191 204</td>
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<td>11</td>
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<td>264 268</td>
<td>271 299</td>
<td>266 281</td>
<td>272 283</td>
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<td>192 268</td>
<td>208 244</td>
<td>253 208</td>
<td>253 279</td>
<td>174 251</td>
<td>226 239</td>
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<tr>
<td>Mean</td>
<td>251 259</td>
<td>248 248</td>
<td>250 249</td>
<td>242 256</td>
<td>232 254</td>
<td>237 244</td>
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<tr>
<td>S.E.M.</td>
<td>18 21</td>
<td>19 22</td>
<td>21 21</td>
<td>19 21</td>
<td>21 23</td>
<td>20 21</td>
</tr>
</tbody>
</table>
FIGURE 2. Urate production by the heart of 13 patients with single left anterior descending coronary artery stenosis, before coronary angioplasty (pre), after each balloon deflation (dilation one to four) and after 15 min of recovery (post). Mean coronary venous-arterial values are given with 1 S.E.M. Significant urate production was found immediately after the last two dilations, and during recovery.

plasma concentration slightly exceeded the venous one before angioplasty (0.58 ± 0.07 and 0.42 ± 0.07 nmol/ml, respectively, \( P = 0.015 \)). Immediately following PTCA these values were 0.32 ± 0.06 and 1.28 ± 0.19 nmol/ml, respectively, \( P < 0.001 \) (average of four attempts). Thus cardiac uptake turned into production. Fifteen minutes after angioplasty arterial plasma hypoxanthine levels were not different from the venous ones.

Discussion

Xanthine oxidoreductase activity is detectable in the heart of a number of species (for reviews, see Schoutsen and De Jong, 1987; Downey et al., 1988). In pig heart it seems to be absent (Podzuweit et al., 1986; Muxfeldt and Schaper, 1987). In rabbit heart both Schoutsen et al. (1983) and Chambers et al. (1985) were unable to demonstrate the enzyme, but Wajner and Harness (1988) measured high activity. The literature on xanthine oxidoreductase in human heart is also conflicting. The reports vary from high (Krenitsky et al., 1974; Jarasch et al., 1986; Wajner and Harness, 1988) to (very) low levels (Watts et al., 1965; Ramboer, 1969; Eddy et al., 1987; Muxfeldt and Schaper, 1987). We want to emphasize that in these reports the number of samples assayed was often very small. Muxfeldt and Schaper (1987) found very low amounts of xanthine oxidoreductase in the two human heart biopsies studied. Krenitsky et al. (1974) reported data on one autopsy sample. These authors observed enzyme activity with ferricyanide as the electron acceptor but did not use NAD or oxygen as the cosubstrate. Allopurinol inhibited the activity. Eddy et al. (1987) could not demonstrate xanthine oxidoreductase in human ventricular tissue. Supposedly the four biopsies studied were not taken from ischemic hearts.

A possible explanation for the discrepancies in activity found could be a difference in quality of the hearts examined. Our data indicate that the enzyme could be active in the human heart in vivo. We cannot exclude that the urate production measured originated from xanthine oxidoreductase activity in polymorphonuclear neutrophils, adhering to areas of the coronary endothelium that are injured by the balloon during inflation.

In the American and Polish studies, mentioned before, blood was deproteinized with HClO₄ which causes a partial loss during sample clean-up. Never the less the arteriovenous differences in urate were significant. Moreover, they suggested that patients with a more severe ischemic heart disease produced the highest amounts of urate. In the American study, patients experiencing pain during a pacing stress test released lactate and showed the highest urate production. Czarnecki (1988) observed that patients with a history of subendocardial infarction produced high amounts of urate whereas patients with normal myocardium or extensive myocardial damage produced less. Our present results support this idea. Group 1, which comprised patients with CCS grades III and IV, showed significant urate production before PTCA. Group 2, in which four out of 13 patients were CCS grade II, only started to produce significant amounts of urate after several dilations. After each of the angioplasty attempts, venous plasma hypoxanthine [the relatively stable substrate for xanthine oxidoreductase (Harkness, 1988)] increased fourfold. The data suggest that ischemic myocardium at risk of infarction produces urate.

Patients of Group 1, all with a proximal stenosis of the left anterior descending coronary artery produced urate (Table 1). It is likely that this urate production was partly
due to endothelial damage, caused by insertion of the guide wire and the balloon catheter. In Group 2 ureate production, which was not significant before PTCA, became obvious after repetitive angioplasty attempts (Fig. 2). Presumably, this is due to cardiac ATP breakdown, with a concomitant rise in hypoxanthine as a result of myocardial ischemia due to coronary occlusion by balloon inflation (see also Serruys et al., 1989). Hypoxanthine serves as a substrate for xanthine oxidoreductase. We suggest that the human heart may contain active xanthine oxidoreductase.

**Acknowledgements**

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**References**


