Leukotriene C₄- and D₄-induced diffuse peripheral constriction of swine coronary artery accompanied by ST elevation on the electrocardiogram: angiographic analysis

Hitonobu Tomoike, M.D., Kensuke Egashira, M.D., Akira Yamada, M.D., Yasuo Hayashi, M.D., and Motoomi Nakamura, M.D.

ABSTRACT Effects of leukotriene (LT) C₄ and LTD₄ on coronary artery spasm in atherosclerotic miniature pigs were examined angiographically. Endothelial balloon denudation of the left circumflex coronary artery was performed in 15 Göttingen miniature pigs; 11 were fed a 2% cholesterol diet and four were fed a regular diet for 3 months. Three months after this denudation, the area of the coronary artery was reduced by 94 ± 2% and 43 ± 5% (p<.01) in the denuded and non-denuded areas by the intracoronary administration of 10 μg/kg histamine after pretreatment with 60 mg/kg iv cimetidine. The effects of LTC₄ and LTD₄ on coronary diameter and the preventive effects of FPL-55712, a LTC₄ and LTD₄-receptor blocker, or diphenhydramine, a histamine H₁-receptor blocker, on histamine-induced coronary spasm were then examined angiographically. Administration of LTC₄ or LTD₄ in doses of 1 and 10 μg injected into the left coronary artery, or selectively into the left circumflex and left anterior descending coronary arteries in a dose of 5 μg, led to the elevations in the ST segment on the electrocardiogram and there was delayed filling of the contrast medium in the peripheral coronary artery. However, these LTs provoked no augmented constrictions at any site on the epicardial coronary arteries (n = 15). Diphenhydramine, 1 mg/kg (n = 6), abolished the histamine-induced coronary spasm. FPL-55712, 0.1 mg/kg, with which the LT-induced myocardial ischemia was abolished, did not prevent the histamine-induced coronary artery spasm. Hence, in a swine preparation of coronary artery spasm, LTC₄ and LTD₄ constricted the coronary arteries at the level of small vessels and rendered the perfused myocardium ischemic. These compounds do not seem to play a primary role in the provocation of epicardial coronary artery spasm induced by histamine in this atherosclerotic swine preparation.


LEUKOTRIENES (LTs), which are 5-lipoxygenase metabolites of arachidonic acid, are released from leukocytes, macrophages, and other cells in response to inflammatory reactions after tissue injury.1, 2 LTC₄ and LTD₄, major active components of the slow-reacting substance (SRS)-A,3 have a potent constrictive effect on vascular smooth muscle.3-6 Accordingly, these LTs are assumed to be one of the causative factors linked to spasm of the coronary artery.4, 6-8 In anesthetized, open-chest intact pigs, dogs, and sheep, intracoronary administration of LTC₄ and LTD₄ reduced coronary blood flow followed by electrocardiographic changes associated with transient ischemia.8-13 Although the LT-induced vasoconstriction led to a marked reduction in coronary blood flow, it has not been clarified whether the LT-induced myocardial ischemia was caused by spasm of the large epicardial vessels or that of the small resistance vessels of the coronary artery.

We designed a miniature swine preparation of coronary artery spasm in which spasm was repeatedly provoked along the atherosclerotic portion of the large epicardial coronary artery in the presence of histamine.14-16 Using this preparation, the goal of the present study was (1) to examine whether LTC₄ and LTD₄ provoke myocardial ischemia with or without coronary artery spasm, and (2) to determine whether LT receptors play any role in evolution of histamine-induced...
coronary artery spasm. To these ends, we used FPL-55712, an antagonist of LTC4 and LTD4. 3, 4, 12, 17, 18

Methods

Animal preparation. Twenty disease-free male Göttingen miniature pigs four to six months old (mean 4.6 ± 1.0) and weighing 13 to 18 kg (mean 15 ± 0.4) were sedated with 12.5 mg/kg im ketamine hydrochloride and anesthetized with 20 mg/kg iv sodium pentobarbital. The pigs were then intubated and ventilated with room air and supplemental oxygen via a positive-pressure respirator (Shinano Inc., Tokyo). Arterial blood pH, PO2, and PCO2 were maintained within normal limits. Under aseptic conditions, a preshaped green Kifa catheter (Kifa, Stockholm) was inserted through the carotid artery into the orifice of the left coronary artery under fluoroscopic guidance. Aortic root pressure was monitored through the Kifa catheter with a Statham P 23 Db pressure transducer. Electrocardiograms were also continuously monitored in limb leads (I, II, III) and in precordial leads (V1, V6). After pretreatment with 5000 units iv heparin, endothelial denudation of the left circumflex coronary artery was performed with a balloon catheter (No. 2 Fogarty Embolectomy Catheter) inserted into the Kifa catheter. 14, 15 Effectiveness of the denudation was confirmed histologically in the five pigs that expired immediately with ventricular fibrillation after the denudation. After denudation, the 11 survivors were fed a laboratory chow diet supplemented with 2% cholesterol and four were fed a regular laboratory chow. The concentration of total plasma cholesterol was measured by an enzymatic method16 before and 3 months after the denudation.

Coronary arteriography and hemodynamic recordings. During the experiment, arterial pressure and electrocardiograms were continuously monitored on a multichannel pen recorder (NEC-Sanei, Polygraph System) and stored on tape with the use of a FM data recorder (R-280 LT, TEAC) for subsequent analysis. Left coronary arteriograms in a left anterior oblique projection were obtained by the manual injection of 5 ml of contrast medium (Urographein 76, Nihon Schering, Osaka, Japan) through the Kifa catheter. Angiograms were obtained with use of a Toshiba 0.6 focal spot x-ray tube on 35 mm cinefilm (CFS 746, Kodak, USA) at 48 frames/sec. The position of the pig and the distance between the pig and image intensifier were kept constant.

Experimental protocol. Three months after the denudation procedure, coronary arteriography was performed without any intervention (control state), after the administration of 20 mg/kg iv nitroglycerin, and after administration of 10 mg/kg ic histamine, which followed pretreatment with 60 mg/kg iv cimetidine in pigs under light anesthesia induced by intramuscular ketamine and intravenous pentobarbital. LTC4 and LTD4 were then injected in doses of 1 and 10 μg, into the left coronary artery of 11 pigs and selectively into the left circumflex or left anterior descending coronary artery of 6 of these 11. In six of the 11 pigs, 10 μg ic LTD4 was also given 2 min after pretreatment with 0.1 mg/kg iv FPL-55712. Subsequently, provocation with 10 μg/kg ic histamine after intravenous cimetidine was repeated. Effects of 1 mg/kg iv diphenhydramine on histamine-induced spasm was tested in the same six pigs. Coronary arteriography was performed 1 and 2 min after the intracoronary administration of histamine and/or the LTs. Injections of histamine and the LTs into the left coronary artery were given through the Kifa catheter after fixation of the catheter position to infuse drugs equally into the left circumflex and left anterior descending coronary arteries. Selective intracoronary administration of the LTs into the left circumflex or left anterior descending coronary arteries was through a radiopaque polyethylene tube inserted into the Kifa catheter.

Pure synthetic LTC4 and LTD4 (kindly supplied by Takeda Chemical Ind., Osaka, Japan) were dissolved in 100% methanol, frozen on dry ice, and stored at -70°C. Stock solutions (100 μg/ml) were diluted with 0.9% saline as a vehicle on the day of the experiment 11. The volume injected was 1.0 ml. A bolus injection of vehicle did not affect the coronary diameter, arterial pressure, or heart rate. FPL-55712 (kindly supplied by Fisons Pharmaceuticals Ltd., U.K.) was dissolved in 0.45% saline 20 and histamine (Sigma Chemical Co., St. Louis) and cimetidine (Fujisawa, Osaka, Japan) were dissolved in 0.9% saline.

Data analysis. Cine films were projected (Tagano 35-cx, Tagano, Horsens, Denmark) and the end-diastolic frame was identified by the R waves of the electrocardiogram recorded on cinefilm. The diameter of the coronary artery was measured with a caliper to 0.05 mm and the absolute diameter (mm) was obtained with the same Kifa catheter, the diameter of which was measured at the end of the study of each animal. The extent of change in diameter of the artery after drug administration was expressed as the percent change in coronary luminal area (mm2) from that after 20 μg/kg iv nitroglycerin to minimize the influence of coronary arterial tone. 21

Reproducibility of the measurements of coronary artery diameter was evaluated by comparing the data measured by individual observers; an excellent correlation between repeated measurements (r = .98, p < .001) and between measurements of different observers was obtained (r = .95, p < .001). To compare quantitatively the responses of the denuded left circumflex and nondenuded left anterior descending coronary arteries to vasoactive agents, two representative sites on each of these arteries were selected to measure the diameter and luminal area throughout the study. The denuded site was the area of the maximal diameter reduction induced by histamine and the nondenuded site was the area of the contralateral coronary artery. In addition, numbers of cine frames between the injection of contrast medium and the visualization of the distal epicardial branches (the third branches) of the left circumflex or the left anterior descending artery were counted in the control state, after intracoronary LTD4, and after LTD4 and pretreatment with intravenous FPL-55712. Since LTC4 and LTD4 are equipotent in constricting the coronary arteries, 3 LTD4 was used in the present study.

Statistical analysis. All results were expressed as the mean ± SE. Statistical significance was tested for by use of Student’s t test. When analysis of variance demonstrated a statistically significant result (p < .05) with respect to sequential data, a Bonferroni’s t test was used to identify subgroup differences. A probability of less than 5% was considered to indicate a statistically significant difference.

Results

Changes in body weight, serum cholesterol, and coronary artery diameter. Five of the 16 pigs died due to ventricular fibrillation just after endothelial denudation. Body weight increased significantly from 15.3 ± 0.2 to 24.0 ± 0.4 kg (p < .01) during 3 months of the experiment and the total plasma cholesterol increased significantly from 54.5 ± 5 to 222 ± 20 mg/dl (p < .01). The coronary artery diameter and luminal area of the left circumflex artery were 2.2 ± 0.1 mm and 4.4 ± 0.6 mm2 and those of the left anterior descending artery were 2.2 ± 0.1 mm and 4.4 ± 0.6 mm2 before denudation (n = 16). Three months after the
Effects of LTs in control miniature swine (n = 4)

<table>
<thead>
<tr>
<th>Coronary area (mm²) and % area reduction (%)</th>
<th>ECG change</th>
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</thead>
<tbody>
<tr>
<td>LCX</td>
<td>LAD</td>
</tr>
<tr>
<td>After nitroglycerin</td>
<td></td>
</tr>
<tr>
<td>LTC₄</td>
<td></td>
</tr>
<tr>
<td>0.1 µg ic IC</td>
<td>4.9±2.0 mm²</td>
</tr>
<tr>
<td>1.0 µg ic IC</td>
<td>4.9±2.0 mm²</td>
</tr>
<tr>
<td>10.0 µg ic IC</td>
<td>4.9±2.0 mm²</td>
</tr>
<tr>
<td>LTD₄</td>
<td></td>
</tr>
<tr>
<td>0.1 µg ic IC</td>
<td>4.9±2.0 mm²</td>
</tr>
<tr>
<td>1.0 µg ic IC</td>
<td>4.9±2.0 mm²</td>
</tr>
<tr>
<td>10.0 µg ic IC</td>
<td>4.9±2.0 mm²</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Absolute luminal coronary area was measured only after intravenous administration of nitroglycerin at a dose of 20 µg/kg. Frequency of ischemic ST changes on the electrocardiogram is shown in the column “ECG change.”

LCX = left circumflex coronary artery; LAD = left anterior descending coronary artery.

denudation, the coronary diameter and area of the left circumflex artery were 2.4±0.1 mm and 4.8±0.8 mm² and those of the left anterior descending artery were 2.5±0.1 mm and 5.0±0.7 mm² (p<.01 vs before denudation; n = 11). Absolute coronary diameters of the left anterior descending artery and the left circumflex arteries did not differ during the same experimental period.

Histamine study. Three months after the denudation and cholesterol feeding, augmented constriction in response to histamine after cimetine was noted at the denuded site (left circumflex artery) in all pigs (n = 11); percent reductions in the luminal area were 94±2% and 43±5% in the left circumflex and left anterior descending arteries, respectively. Arterial pressure decreased significantly by 10 to 20 mm Hg 30 sec after histamine and almost recovered to the control level by around 60 and 120 sec, when coronary arteriography was performed. Heart rate increased slightly after histamine and returned to control levels during coronary arteriography. Significant ST segment elevation, defined as ST segment shifts from the control level of over 0.1 mV, occurred at the related site (in leads of II, III, and V₆) of the denuded coronary artery in six of 11 pigs. There was a significant difference in coronary constriction in the group with and that without ST elevation (66±4% and 80±7% diameter constriction, respectively.)

Effects of LTs. Arterial pressure did not change significantly 1 and 2 min after LTC₄ and LTD₄, 1 µg into the left main coronary artery, or LTD₄, 5 µg into the left anterior descending or left circumflex artery, but it increased significantly after 10 µg of the LTs was injected into the left main coronary artery. Heart rate did not change significantly during the period of angiography. Dose-dependent electrocardiographic ST elevations were noted 60–100 sec after intracoronary administration of LTs in all leads (tables 1 and 2), anterior and posterior, after left main, left anterior descending, and left circumflex artery injections, respectively (figure 1). LTC₄ and LTD₄ in the doses given constricted equally both the denuded and non-denuded areas of the epicardial coronary artery by less than 40% and augmented vasoconstriction was not induced at any site on the large epicardial coronary arteries in either control or atherosclerotic swine (figures 2 and 3; tables 1 and 2). Intervals between the time of injection of contrast medium into the left main coronary artery and the visualization of the third branches of the epicardial coronary arteries in both the left circumflex and left anterior descending arteries were significantly prolonged after administration of LTs com-

TABLE 2

Effects of LTs in atherosclerotic miniature swine (n = 11)

<table>
<thead>
<tr>
<th>Coronary area (mm²) and % area reduction (%)</th>
<th>Heart rate (beats/min)</th>
<th>mAoP (mm Hg)</th>
<th>ECG change</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCX</td>
<td>LAD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After nitroglycerin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ 60 mg/kg iv cimetidine</td>
<td>4.8±0.8 mm²</td>
<td>5.0±0.7 mm²</td>
<td>136±10</td>
</tr>
<tr>
<td>LTC₄, 1 µg IC</td>
<td>28±6%</td>
<td>16±4%</td>
<td>144±9</td>
</tr>
<tr>
<td>LTC₄, 10 µg IC</td>
<td>20±4%</td>
<td>16±5%</td>
<td>144±8</td>
</tr>
<tr>
<td>LTD₄, 1 µg IC</td>
<td>20±5%</td>
<td>18±3%</td>
<td>141±8</td>
</tr>
<tr>
<td>LTD₄, 10 µg IC</td>
<td>15±4%</td>
<td>8±3%</td>
<td>143±8</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Absolute luminal coronary area was measured only after intravenous administration of nitroglycerin at a dose of 20 µg/kg. ECG change represents number of 11 cases with ischemic electrocardiographic ST changes.

mAoP = mean aortic pressure; LCX = left circumflex coronary artery; LAD = left anterior descending coronary artery.

₈p < .01 vs LAD; ₉p < .05; ₁₀p < .01 vs after nitroglycerin.
pared with intervals in the control angiograms (table 3). Such LT-induced ST elevations and the delayed filling of the third branches of the epicardial coronary arteries with contrast were suppressed after pretreatment with 0.1 mg/kg iv FPL-55712 (figure 2; table 3).

**Prevention of histamine-induced coronary spasm.** After administration 0.1 mg/kg iv FPL-55712 and 1 mg/kg iv diphenhydramine, arterial pressure and heart rate did not change significantly. As shown in figure 4, FPL-55712 failed to prevent the histamine-induced constriction both at the denuded site (left circumflex artery) and the nondenuded site (left anterior descending artery) in all six pigs. Diphenhydramine, 1 mg/kg iv, a histamine $H_1$-blocker, prevented histamine-induced constriction in the same six pigs (table 4).

**Discussion**

Using miniature pigs and coronary arteriography, we examined the effects of leukotrienes LTC$_4$ and LTD$_4$ on coronary artery spasm. The present study
FIGURE 3. Coronary angiograms obtained after injections of LTD₄ at various sites. A, Control; B, injection of 10 µg LTD₄ into the left main coronary artery; C, injection of 5 µg LTD₄ into the left anterior descending coronary artery; D, injection of 5 µg LTD₄ into the left circumflex coronary artery. Arrow indicates the site of dye filling delay. These angiograms were taken in the same heart as in figure 2.

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A significant reduction in coronary blood flow and associated electrocardiographic signs of ischemia after intracoronary injection of LTC₄ and LTD₄ have been noted in the intact pig heart. However, the site of coronary artery contraction that is critical for the development of myocardial ischemia has not been clarified. In a study using the cheek pouch, LTC₄ and LTD₄, applied topically for a 3 min period in 0.3 to 20 nM concentrations elicited an intense dose-dependent contraction of arterioles, in particular the terminal arterioles. Since augmented coronary contraction was provoked along the segments of the epicardial coronary artery in our swine and in canine preparations, we applied coronary arteriography to visualize the entire epicardial coronary arterial trees and to determine the site of focal spasm without thoracotomy and without dissecting the artery.

Results of the present study confirmed our previous findings that the arteriosclerotic portion of the coronary artery responded vigorously to H₁ stimulation. These findings were in agreement with augmented vascular responses to histamine of human epicardial coro-
TABLE 4
Prevention of histamine-induced spasm in miniature swine (n=6)

<table>
<thead>
<tr>
<th></th>
<th>LCX</th>
<th>LAD</th>
<th>Heart rate (beats/min)</th>
<th>mAoP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After nitroglycerin</td>
<td>4.8 ± 1.1 mm²</td>
<td>5.0 ± 1.8 mm²</td>
<td>140 ± 15</td>
<td>96 ± 4</td>
</tr>
<tr>
<td>Cimetidine iv + histamine ic</td>
<td>94 ± 4%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43 ± 5%</td>
<td>134 ± 12</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>FPL iv + cimetidine iv + histamine ic</td>
<td>94 ± 2%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40 ± 5%</td>
<td>143 ± 10</td>
<td>95 ± 3</td>
</tr>
<tr>
<td>Diphenhydramine iv + cimetidine iv + histamine ic</td>
<td>16 ± 3%&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>8 ± 2%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>152 ± 8</td>
<td>105 ± 7</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Absolute luminal coronary area was measured only after intravenous administration of nitroglycerin at a dose of 20 µg/kg.

mAoP = mean aortic pressure; LCX = left circumflex coronary artery; LAD = left anterior descending coronary artery.

<sup>a</sup>p < .05 vs LAD; <sup>b</sup>p < .05 between control study and FPL study.

Coronary arterial strips with atherosclerosis<sup>24</sup> and histamine-induced angina in patients with variant angina.<sup>25</sup> Accordingly, we consider this preparation useful for the analysis of the pathophysiology of coronary artery spasm.

Experiments conducted in isolated guinea pig hearts (Langendorff preparation) have shown that LTC<sub>4</sub> and LTD<sub>4</sub> are potent constrictors of the coronary artery.<sup>5,6</sup> LTs administered selectively into the coronary arteries in situ of anesthetized domestic pigs,<sup>8,9,17</sup> sheep,<sup>12</sup> and dogs,<sup>10,11,13</sup> reduced the coronary blood flow without systematic effects. In the present study, in which LTC<sub>4</sub> and LTD<sub>4</sub> were administered in vivo into the left coronary artery of pigs, myocardial ischemia occurred in the related areas not only in animals with histamine-induced coronary artery spasm but also in normal pigs. This ischemia was invariably associated with delayed filling of the contrast medium in the peripheral coronary vasculature. Thus, the preferential site of arterial contraction may be the terminal arterioles, as was noted in the study using the cheek pouch.<sup>26</sup> These LT-mediated small vessel constrictions were unaffected after coronary denudation.

In the present study, enhanced contraction of the large coronary artery similar to that observed after histamine was not demonstrated angiographically at any site on the left circumflex (denuded site) or the left anterior descending coronary artery (nondenuded site), after the intracoronary administration of the LTs. Furthermore, FPL-55712 in an adequate dose to prevent LT-induced myocardial ischemia did not modulate the extent of histamine-induced constrictions. Thus, it was suggested that in our swine preparation LTC<sub>4</sub> and LTD<sub>4</sub> did not induce augmented contraction of the epicardial coronary artery, but rather these compounds caused myocardial ischemia as a result of enhanced

![FIGURE 4. Coronary angiograms and electrocardiograms obtained in the control state, after 10 µg/kg ic histamine with intravenous cimetidine, and after pretreatment with 0.1 mg/kg iv FPL-55712. Note that histamine-induced spasm (arrow) was not attenuated by FPL-55712.](image)
constriction of the small vessels. This evidence contradicts the data obtained in studies with tracheal smooth muscle, in which the tracheal insuffluation pressure in anesthetized artificially ventilated pigs increased at least 100 times more with LTC4 compared with the increase after histamine.20 Accordingly, in the relative potency of smooth muscle contraction induced by LTs and histamine, there was a large difference among species,27 organs,27 locus of arteries,22 and arteries with and without vasoospastic characteristics, as shown in the present study. Mechanisms of these difference may be related to the functional state of LT receptors,28 but further work with regard to LT receptors in coronary arteries has to be done.

In our swine preparation of coronary artery spasm, LTs (LTC4 and LTD4) did not play a primary role in the provocation of coronary artery spasm. However, Mullane et al.29 noted an increase in lipoxygenase products in the infarcted area and significant protection of the myocardium by the relatively selective lipoxygenase inhibitor BW 755C after coronary occlusion of the canine heart. Thus, it is possible that the LT-induced myocardial ischemia associated with histamine-induced coronary spasm may result in severe myocardial ischemia due to the extensive contraction of coronary arteries from the conduit to the level of small vessels.

Lee et al.30 suggested that a diet enriched with fish oil-derived fatty acids has anti-inflammatory effects because it inhibits the 5-lipoxygenase pathway in neutrophils and monocytes and the LT-B4-mediated functions of neutrophils. However, effects of a high-cholesterol diet on LT receptors have not been examined. In the present study, LTC4 and LTD4 induced similar electrocardiographic ST change in groups on a regular diet and those consuming 2% cholesterol. Thus, LT receptors on coronary artery smooth muscle may not be altered by a diet with 2% added cholesterol.

Direct extrapolation of these findings in pigs to patients with coronary spasm and/or ischemic heart disease may not be feasible, since vascular responses to the LTs differ among species.27 However, our observations do pave the way for elucidation of the pathophysiology of coronary artery spasm.

We thank Dr. H. Shimokawa for discussions, Dr. S. Terao of Takeda Chemical Ind. for the generous supply of LTC4 and LTD4, and pertinent discussion, Fisons Pharmaceuticals Ltd. for FPL-55712, K. Kobayashi, T. Kawasaki, R. Satoh, and K. Shozaki for technical assistance, M. Ohara for comments on the manuscript, and Y. Uchiyama and I. Tomizuka for secretarial services.

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Leukotriene C4- and D4-induced diffuse peripheral constriction of swine coronary artery accompanied by ST elevation on the electrocardiogram: angiographic analysis.
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Circulation. 1987;76:480-487
doi: 10.1161/01.CIR.76.2.480

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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