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Protective effect of lidocaine in the experimental foot-and-mouth disease pancreatitis

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Abstract. Experimental infection of mice with foot-and-mouth disease virus (FMDV) induces a necrotizing pancreatitis of the exocrinar portion of the organ. The lesions are characterized by vascular congestion, edema and interstitial polymorphonuclear leukocyte (PMN) infiltrates. When infected mice were treated with different amounts of lidocaine (a local anesthetic, chemically defined as a tertiary amide compound), reduction in intensity of the pancreatic necrosis and in the number of PMN were observed. Even though lidocaine could interfere with FMDV post-replicative cytolytic mechanisms, it appears that protection against pancreatic necrosis is by attenuation of PMN presentation in the infected tissue.
Key words. Foot-and-mouth disease; lidocaine; pancreatitis.

Mice and guinea pigs are the laboratory animals that contribute most to the knowledge of experimental foot-and-mouth disease (FMD)[1,2]. Several lesions have been described in newborn mice, varying depending on the mouth strain, the virus strain employed and the inoculation site used[3]. In pregnant female mice lesions have been described mainly in the pancreas, skeletal muscle and myocardium[4]. In intraperitoneally (i.p.)-infected adult mice, the only lesion found was an acute pancreatitis with edema, interstitial infiltration of polymorphonuclear leukocytes (PMN), and massive necrosis of the exocrinar pancreas. The pathological alterations do not produce external clinical signs. Furthermore, they last for less than a week[5].

At the present time, the pathogenesis of the pancreatic necrosis induced by FMD virus (FMDV) in the murine model is still under analysis. It is possible to show (article in preparation) the presence of FMDV antigenic determinants in the pancreatic tissue 6 to 12 hours postinfection (p.i.) by means of immunohistochemical studies; however, edema and PMN infiltrates in the connective-vascular interstitium are the first morphological alterations, the latter occuring before necrosis of the tissue. Lidocaine is a local anesthetic, chemically defined as a tertiary amide compound. Amongst other effects, the drug has a particular activity on several cells and molecules that participate in the acute inflammatory response[6–8].

We present here evidence of the anti-inflammatory effect of lidocaine administered to adult mice experimentally infected with FMDV.

Materials and methods

Animals. Adult outbreeding 60-day-old male Swiss mice were used.

Virus. An O_1 Caseros strain of FMDV adapted in mice was used. Briefly, the viral strain was maintained for several passages on BHK_{21} cell monolayers, and frozen in aliquots at −70 °C. Adaptation of the virus to mice was obtained by i.p. inoculation of 1000 $TCID_{50}$ of FMDV to adult mice. Blood samples were collected every 48 h. Other adult mice were inoculated by an i.p. route with this blood. After the third passage, the pancreas of the mice were homogenized with 10% Eagle's MEM (w/v). The virus present in the supernatant obtained from centrifugation of the homogenate was titered on BHK_{21} cells. Viral titer used for our experiments was 10^6 $TCID_{50}$/ml.

Chemicals. Lidocaine Chlorhydrate 2% (Xylocaina® ASTRA).

Simultaneous viral infection and administration of lidocaine. Four groups of mice were inoculated i.p. with FMDV (10^3 $TCID_{50}$). The animals from the experimental group were given different doses of lidocaine i.p. (group A = 0.08 mg/g body weight (8.5 mM); B = 0.16 mg/g (17 mM); C = 0.39 mg/g (42 mM)) at the infection time, and subsequently every 8 h. The control group received only 0.1 ml of saline. The final administration of lidocaine was performed at 40 h p.i. Four mice from every group were sacrificed at 48 h p.i. The remaining mice were sacrificed at 7 days p.i. for determination of the infection course.

Administration of lidocaine before and after FMDV infection. Mice were separated into three groups. All the animals were infected with FMDV (10^3 $TCID_{50}$) by an i.p. route. Group A mice received 0.16 mg/g of lidocaine 8 h prior to the viral infection. Group A and B mice were treated with 0.16 mg/g lidocaine every 8 h after infection, until 40 h p.i. Group C mice received the first 0.16 mg/g dose of lidocaine at 24 h p.i. and then every 8 h up to 40 h p.i. All the animals were killed at 48 h p.i.

Table 1. Intraperitoneal treatment of FMDV-infected mice with lidocaine.

Group	Lidocaine	Cong/exud[a]	PMN infiltrate[b] (%)	Necrosis[c] (%)
A	0.08 mg/g	++++	50–60	20–30
B	0.16 mg/g	+++	20–30	10–20
C	0.39 mg/g	++	10–20	0–5
D	-	++++	100	80–90

FMDV-infected mice were treated with different amounts of lidocaine. [a] The cross indicates the magnitude of the vascular congestion and seric exudate. [b] The percentage PMN indicates the proportion of pancreas infiltrated with those cells. [c] The percentage necrosis illustrates the proportion of exocrinar tissue affected.

Table 2. Intraperitoneal pretreatment of FMDV-infected mice with lidocaine.

Group	Treatment (h)	Cong/exud[a]	PMN infiltrate[b] (%)	Necrosis[c] (%)
A	−8	+	10–20	0–5
B	0	+++	20–30	10–20
C	+24	++++	40–50	20–30

Mice were treated with 0.16 mg/g body weight of lidocaine at different times with respect to the time of infection (0 h). The drug was then administered every 8 h. The animals were killed at 48 h p.i. [a]The cross indicates the magnitude of the vascular congestion and seric exudate. [b]The percentage PMN indicates the proportion of pancreas infiltrated with those cells. [c]The percentage necrosis illustrates the proportion of exocrinar tissues affected.

Table 3. Blood and pancreatic titers of FMDV-infected mice.

Sample	Days p.i.	Treated mice	Untreated mice
Pancreas	2	$2.6 \pm 0.35 \times 10^3$	$3.4 \pm 0.75 \times 10^3$
	7	NVD	NVD
Blood	2	NVD	NVD
	7	NVD	NVD

Mice were infected with 10^3 $TCID_{50}$ FMDV and killed at 2 or 7 days p.i. Pancreatic titers (expressed in $TCID_{50}/g$) were determined on BHK_{21} cell monolayers after 36 h incubation at 37 °C in a 5% CO_2 atmosphere. NVD = No virus detected.

Titration of FMDV in pancreas of infected mice. Each of three pancreas obtained from bled mice at 48 h p.i. was homogenized in 10% Eagle's MEM (w/v). Suspensions were centrifuged at 25,000 *g* for 30 min at 4 °C, and tenfold dilutions of the supernatant were absored on BHK_{21} cell monolayers quadruplicate for 1 h at 37 °C. Tenfold dilutions of the heparinized blood were also titered on the same cells. Microtiter cell well plaques were stained with crystal violet 1% 48 h later. Viral titer was determined by the Reed and Muench analysis[9] and expressed as $TCID_{50}/g$ (pancreas) or $TCID_{50}$/ml (blood).

Histopathology of FMDV-infected mice. The pancreas of the killed mice in all the experiments were fixed in buffered formaldehyde 10%, embedded in paraffin, cut at 5 μm thickness and stained with hematoxylin and eosin.

The intensity of PMN infiltration and of pancreatic necrosis was expressed as the percentage of the tissue affected in all of the semiserial cuts of the whole organ.

Results

Treatment at time of infection. During treatment with lidocaine, none of the infected or control group mice showed any apparent clinical sign except for the C group mice, where a 30 min prostration and recovery after the drug administration were observed. The only lesion found was an acute pancreatitis of the exocrinar portion without involvement of the islets of Langerhans.

The pancreas of non lidocaine-treated mice showed vascular congestion, edema and interstitial PMN infiltrates (table 1). The edema separated the pancreatic lobes, inducing a disturbance in the normal structure of the organ. Signs of cellular necrosis were observed, including cytoplasmic swelling and eosinophilia, pyknosis and the disappearance of secretory granules. The group A mice (treated with 8.5 mM lidocaine) showed vascular congestion and interstitial edema within a PMN infiltrate, and necrosis of 20 to 30% of the pancreatic acini. In the group B mice (treated with 17 mM lidocaine) vascular congestion and edema were significantly reduced, as was the PMN infiltration and the acinar necrosis present in no more than 20% of the tissue. Animals receiving 42 mM lidocaine (group C) produced a mild interstitial exudate consisting of a soft edema and a few PMN. Necrosis of acinar cells was observed in less than 10% of the parenchyma. Restitution 'ad integrum' of the pancreas was observed in those mice killed at 7 days p.i.

Pre and postinfection treatment. Data given in table 2 show that treatment with lidocaine before the FMDV infection noticeably reduces the lesions observed in the infected animals in comparison with those that received the drug simultaneously with the virus or those that were treated after infection.

Titration of FMDV in pancreas of infected mice. Results given in table 3 do not show significant differences between lidocaine-treated and non-treated mice. FMDV could not be detected in the blood samples analyzed, and was only detected in the pancreatic samples obtained at 48 h p.i.

Discussion

Experimental infection of mice with FMDV produces a necrotizing pancreatitis of the exocrinar portion of the organ. Chronology of microscopic lesions found were described elsewhere[5].

The cytolytic capacity of FMDV in tissue culture, the speed of appearance of pancreatic cellular necrosis, the

progressive accumulation of viral antigens in those cells, and the correlation of viral infectivity with the severity of the tissue damage, suggest a cytolytic viral replication. This assumption seems to be only partially true since treatment with lidocaine reduced the intensity of the pancreatic necrosis and the number of PMN without modifying the concentration of virus either in the tissue or in the peripheral blood. Nevertheless, this cannot exclude any effect of FMDV on post-replicative cytolytic mechanisms.

Polymorphonuclear leukocytes have been proposed as effector cells with cytotoxic activity on several virus-infected cells in the absence of antibodies and complement in vitro[10]. Lidocaine, as well as other local anesthetics, has a depressive activity on macrophage functions such as adherence, chemotaxis, phagocytosis, degranulation and respiratory burst[6,7], post-phagocytic oxygen consumption and superoxide anion production[11,12]. A probable anti-inflammatory activity has recently been proposed due to its effect on proinflammatory cytokines and PMN adhesion molecules[8].

The way the experiments were performed in this work may not establish an unequivocal activity of lidocaine. However, the most marked observation is the sharp reduction in the PMN infiltration in the pancreas of treated animals which coincides with the marked reduction in the tissue necrosis. This phenomenon shows a linear correlation with the drug concentration and frequency of administration. Given before or simultaneously with infection, lidocaine induced a greater protective effect against tissue damage than when given later (24 h p.i.). Similar behavior, although not as clear, was observed in 42 mM lidocaine-treated mice.

In most cases of spontaneous human and animal pancreatitis, as well as in experimentally-induced pancreatitis, activation of pancreatic proenzymes can be observed. This activation induces the appearance of vascular lesions, hemorrhages, cytosteatonecrosis, and peripancreatic compromise[13,14]. In our model, FMDV-induced lesions were characterized by a necrosis confined to acinar and ductal pancreatic cells, without any other type of organic lesion or sign of illness, and ending with the restitution 'ad integrum' of the pancreas. These results lead us to conclude that participation of pancreatic enzymes has relatively little influence on the pathogenesis of tissue damage. Therefore, it does not seem necessary to speculate on the effects of lidocaine at that level. The observations described suggest that tissue necrosis begins with lytic viral replication which is increased by the PMN activity. Therefore, it is tempting to propose that lidocaine has at least a partial protective effect against pancreatic necrosis by means of an attenuation of PMN presentation in the infected tissue.

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