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Short Communication

Enhancement of murine susceptibility to oral lactate dehydrogenase-elevating virus infection by non-steroidal anti-inflammatory agents, and antagonism by misoprostol

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Summary

The murine lactate dehydrogenase-elevating virus (LDV) was used to study the effects of prostaglandin-acting agents on mucosal resistance to virus infection. Mice treated with non-steroidal anti-inflammatory drugs (NSAIDs) prior to oral exposure to LDV demonstrated a reduction in the mucosal barrier to LDV infection. Histological studies indicated that these NSAID effects were not a result of gross or microscopic tissue damage. The effects of two NSAIDs, indomethacin and diclofenac, were inhibited by co-treatment of mice with misoprostol, a synthetic PGE_1 analog. The ability of misoprostol to modulate NSAID effects was not due to direct antiviral activity or to actions on LDV-permissive macrophages. These results show that the mammalian mucosal barrier to virus infection is prostaglandin-sensitive, and provide a model for the study of resistance to viral infection.

Virus infection; Mucosal barrier; Misoprostol; NSAIDs

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Exposure to viruses as well as other infectious agents commonly occurs at sites lined by mucous membranes, which contain non-specific host defenses to infection (Braude, 1981). Biochemical as well as physical properties of this mucosal barrier may act to inhibit the ability of virions to infect host target cells. Thus, the mere presence of virus within a potential host does not necessarily lead to infection, which is dependent upon exposure to a minimum infectious dose of virus (Ward and Akin, 1984). Although these principles are clear, the mechanisms which regulate the infectivity of individual viruses are not well understood.

Lactate dehydrogenase-elevating virus (LDV) is a murine togavirus which infects macrophages (Rowson and Mahy, 1985; Cafruny, 1989). The first stage in the infectious process is receptor-mediated attachment of the virus to a permissive host target cell (Kowalchyk and Plagemann, 1985). The existence of a powerful relative mucosal barrier to LDV infection has been demonstrated in orally-exposed mice (Cafruny and Hovinen, 1988): whereas one virion is sufficient to cause productive infection in parenterally exposed mice, a dose of greater than 100 000 virions is required to cause infection by the oral route. Thus, LDV provides an important model for the study of the non-specific mucosal barrier to viral infection, and results obtained with LDV may have significance for other viruses which are more difficult to study in the laboratory but which have similar cellular tropisms.

The present work describes the ability of non-steroidal anti-inflammatory drugs (NSAIDs) to weaken the mucosal barrier to LDV infection in drug-treated mice subsequently exposed to LDV by the oral route. The effects of some NSAIDs were found to be modified by cotreatment of the mice with the cytoprotective agent misoprostol, a synthetic prostaglandin E1 analog (Bauer et al., 1986).

Since the minimum infectious dose of LDV is about $10^{5.3}$ ID_{50} orally (Cafruny and Hovinen, 1988), we hypothesized that a higher dose of LDV could be used to infect groups of mice with a fractional rate of infection. Initial titration experiments (data not shown) confirmed that an oral dose of LDV which infected a reproducible fraction of mice could be devised. This dose, which we have termed a limiting dose since not all mice in an exposed group are infected, was approximately 10^7 virions, and could be administered either in drinking water or by pipet. The selected rates of infection with limiting doses varied between about 10–40% for individual virus pools, were stable for virus storage periods of up to 6 months, and thus provided a value for which treatment-induced enhancement or suppression could be observed. There was no evidence of delayed infection, as determined by re-assay of mice at 1–2 weeks post exposure, and the mice, which were housed under standard conditions, remained healthy in appearance throughout the experiments.

Data for the first two experiments, in which mice were exposed to LDV in drinking water, are shown in Table 1. When groups of mice were treated with indomethacin (0.6 mg given in two doses of 0.45 and 0.15 mg at 3.5 and 0.5 h prior to LDV exposure, respectively) or aspirin (1.0 mg given 2 h prior to LDV exposure), the rates of observed infection relative to those in normal control mice were significantly enhanced. Thus, these two NSAIDs appeared to reduce the mucosal barrier to LDV infection, since more mice became infected following NSAID treatment, as indicated by the percent change in Table 1. As seen in the data from experiment 2, misoprostol, a synthetic PGE_1 analog with known cytoprotective

TABLE 1

Effects of indomethacin, aspirin, and misoprostol on oral LDV infectivity

Expt.	Drug	No. mice	Percentage	
			Infected	Change
1	Vehicle	82	34	
	indomethacin 0.6 mg	82	77	+126, $P<0.01$
	aspirin 1 mg	17	88	+159, $P<0.01$
2	Vehicle	36	42	
	indomethacin 0.6 mg	42	81	+93
	indomethacin 0.6 mg + misoprostol 7.5–10 μg	44	59	+40, $P<0.04$

Female 20–28 g outbred NIH mice (Amitech, Inc., Omaha) were fasted for 12–16 h, and exposed to about 10^7 ID_{50} LDV-P (Cafruny and Plagemann, 1982) in drinking water overnight (12–16 h, Cafruny and Hovinen, 1988). NSAIDs prepared as stock solution in ethanol and diluted in pH 7.4 PBS were given orally in the total doses indicated. The NSAID doses and their timing relative to LDV exposure were based on previous NSAID effects on rat gut (Bauer et al., 1986): 0.45 and 0.15 mg indomethacin were given at 3.5 and 0.5 h prior to LDV, respectively; and aspirin was given 2 h prior to LDV. The rate of LDV infection was determined by measuring blood lactate dehydrogenase concentrations at 4–5 days post-exposure (Plagemann et al., 1963). Misoprostol diluted from ethanol stocks in pH 7.4 PBS was administered in 3–4 doses (oral or peritoneal) of 2.5 μg each, spaced 1.5–4.5 h prior to LDV exposure. A transient diarrheal response was noted as evidence of successful drug delivery (personal communication, Dr W. Perkins, G.D. Searle and Co.). Significant differences were determined by the Student's t-test, applying the arcsine transformation (Sokal and Rohlf, 1969), and are indicated for NSAIDs relative to background rate in mice receiving buffered ethanol (vehicle), and for misoprostol relative to NSAID-treated mice.

effects (Bauer et al., 1986; Herting and Nissen, 1986), antagonized the effect of indomethacin on the rate of LDV infectivity. The rate of infection fell significantly from 81% to 59% in mice receiving both indomethacin and misoprostol.

Table 2 shows the data from experiments 3–8, in which mice were exposed to LDV by mouth pipet. As shown in Table 2, control rates of infection (6–14%) were less than those in experiments 1 and 2, but this was not necessarily due to the switch to pipet-administered virus, since the virus pools used in experiments 3–8 were different from those used in the previous experiments. Aspirin, diclofenac and ibuprofen significantly enhanced the rate of LDV infectivity, and their effects appeared to be relatively independent of the background (control) rate of infection. However, misoprostol, while displaying activity against diclofenac (20% infection versus 34% in diclofenac-only treated mice), had no effect on the aspirin response, as seen in experiments 3 and 4. Interestingly, when misoprostol was tested against ibuprofen in experiment 8, it enhanced the effect of ibuprofen. Thus, based on the infectivity rate of mice receiving both an NSAID and misoprostol, there was separation of NSAIDs into three groups according to the response to misoprostol.

In order to study the direct effects of misoprostol on LDV, primary macrophage cultures were pretreated with misoprostol prior to in vitro LDV infection (Cafruny et al., 1986). Experiments were carried out in both serum-free medium and medium supplemented with 1% fetal bovine serum, using a high multiplicity of infection (500–1000). Misoprostol, added to cultured macrophages at 50 μg/ml 4 h prior

TABLE 2

Effects of aspirin, diclofenac, ibuprofen and misoprostol on oral LDV infectivity

Expt.	Drug	No. mice	Percentage	
			Infected	Change
3	None	42	14	
	Vehicle	68	18	
	Misoprostol 30 μg	68	10	
	Aspirin 2 mg	68	30	+114, $P<0.05$
	Aspirin 2 mg + misoprostol 30 μg	68	32	+129
4	Vehicle	66	6	
	Aspirin 2 mg	66	17	+183, $P<0.05$
	Aspirin + misoprostol 10 μg	57	18	+200
5	Vehicle	82	11	
	Diclofenac 3.6 mg	99	34	+209, $P<0.01$
	Diclofenac 3.6 mg + misoprostol 10 μg	71	20	+82, $P<0.04$
6	None	112	12	
	Ibuprofen 3.6 mg	147	15	+25
7	Vehicle	60	10	
	Ibuprofen 7.2 mg	60	33	+230, $P<0.01$
8	Ibuprofen 7.2 mg	108	44	
	Ibuprofen + misoprostol 10 μg	110	67	+52, $P<0.01$

Female 20–28 g outbred NIH (Amitech, Inc., expts. 3–5) or CF1 (Sasco, Inc., Omaha, expts. 6–8) mice were fasted for 12–16 h prior to receiving an oral dose of about 10^7 ID_{50} LDV given by pipet in a volume of 10–30 μl. Other methods are as described in Table 1. Ibuprofen and diclofenac were given in two equal doses, 4.5 and 2.5 h prior to LDV. Significant differences are indicated for NSAIDs relative to the background rate of infection in control mice, and for misoprostol relative to NSAID rates.

to LDV infection, and replenished at 10 μg/ml at the time of infection, had no significant effect on the percentage of LDV infected macrophages in either experiment: the percentage of cells scoring positive for LDV infection in serum-free medium was 3.3% in control cultures and 3.4% in misoprostol-treated cultures; medium supplemented with 1% serum yielded infection rates of 6.5% (control) and 8.0% (misoprostol-treated). Thus, the modulating effects of misoprostol on LDV infectivity after NSAID treatment were non-specific on the mucosal barrier to infection, rather than on the viral-permissive cell itself.

Since NSAIDs may produce gastric lesions in rats (Bauer et al., 1986), we considered the possibility that NSAID enhancement of the rate of LDV infection was due to drug-induced GI hemorrhage or ulceration. Therefore, 3–5 mice from each of the NSAID treatment groups, as well as misoprostol-treated and vehicle-treated mice (total of 40 mice), were sacrificed for histological studies.

Grossly, the entire GI tract from NSAID or misoprostol-treated mice appeared normal, with no signs of hemorrhage or ulceration. Microscopical analyses of formalin-fixed sections of esophagus, stomach, and small intestine failed to reveal any evidence of cellular inflammation or disruption. However, the stomachs of several mice each from groups of 5 mice treated with diclofenac or ibuprofen

contained infrequent minute mucosal necroses (one lesion every 1–4 mm), which were absent in stomachs of mice treated with ibuprofen + misoprostol. These necroses were not superficial, but occurred deep in the mucosa adjacent to the muscularis mucosa. The histological data indicate that the mechanism of NSAID actions on the mucosal barrier to LDV is not via hemorrhage or superficial damage. However, they also suggest that misoprostol may inhibit development of minute necrotic lesions in the mouse stomach, and further studies of this potential effect of misoprostol seem warranted.

These results are significant in describing a viral model of infectivity in which drug effects on mucosal resistance can be determined. Our data show that indomethacin, aspirin, ibuprofen, and diclofenac inhibit the mucosal barrier to oral LDV infection, as determined by their effects on the rate of infection during exposure to a limiting virus dose. These results suggest that a general property of NSAIDs may be to inhibit viral defense mechanisms at gastrointestinal sites. It is important to point out that the drug dosages used in these experiments were considerably higher than those generally administered to human patients, so we view these data to be primarily of interest to the study of mechanisms of viral protection within the GI tract, rather than a demonstration of clinically significant NSAID effects. Nevertheless, it cannot be ruled out that NSAIDs might have a medically important effect on viral defense of humans, particularly when other factors are involved.

The mechanism of NSAID action in the LDV system is not clear, but is not related to suppression of specific host immunity, since the mice were not immunized to LDV prior to exposure, and mice have no natural immunity to the virus (Rowson and Mahy, 1985; Cafruny, 1989). Thus, we conclude that NSAIDs inhibit either physical or chemical aspects of the non-specific mucosal barrier to infection. The GI site within which LDV infection is initiated following oral exposure is not known. Delivery of virus to the stomach results in a 100-fold increase in sensitivity to infection (data not shown), supporting the possibility that the stomach and small intestine are sites of infection. Thus, LDV might be an important model for a variety of enteric viruses, including those with a lower MID than LDV.

Since LDV infectivity is most efficient during parenteral exposure, hemorrhage of the GI tract was considered as a potential mechanism for NSAID effects on the rate of infection. However, examination of the esophagus, stomach, and small intestine of mice exposed to NSAIDs failed to reveal any gross evidence of tissue damage, hemorrhage, or ulceration. The absence of microscopic lesions in tissues from drug treated mice, despite the rare minute necroses noted in some stomachs of diclofenac and ibuprofen-treated mice, also argues against a hemorrhagic NSAID mechanism in this mouse model. Thus, we conclude that the effects of NSAIDs on the mouse GI tract are likely to be ultrastructural in nature, through discrete ultrastructural changes in the viral protective mechanism.

That misoprostol is able to protect against the deleterious effects of indomethacin and diclofenac on LDV susceptibility demonstrates another category of cytoprotection which may be ascribed to this drug. Although the mechanism of action is unknown at present, misoprostol has been previously shown to regulate gastric bleeding (Hunt et al., 1983), suggesting that a hemodynamic effect in the GI tract

could play a role in inhibition of NSAID actions. Misoprostol also inhibits gastric acid secretion, but this effect would not be expected to protect against LDV, which is acid-sensitive (Crispens, 1965). In previous studies, prostaglandins and prostaglandin analogs have been shown to tighten the gastric barrier to diffusion of acid (Dajani et al., 1978; Chaudhury and Jacobson, 1978), and prostaglandins have been shown to stimulate the release of mucus and mucus synthesis (Bolton et al., 1978; Kaymakcalan et al., 1984; Tao and Wilson, 1984). Furthermore, misoprostol stimulates mucosal secretion (Wilson et al., 1986) as well as the thickness of the gastric mucus layer (Sellars et al., 1986). Thus, a beneficial effect of misoprostol on the NSAID-damaged mucus layer may account for inhibition of LDV infectivity in misoprostol and NSAID-treated mice.

It is not clear why aspirin failed to respond to misoprostol or why misoprostol appeared to synergistically act with ibuprofen in suppressing the mucosal barrier to LDV. It is possible that optimal dosages of misoprostol were not given in these experiments, and future studies of dose and timing of misoprostol might be of interest. The separation of NSAIDs into three distinct classes suggests that different drug interactions may be important in the observed effects on LDV infectivity, and also that effects secondary to NSAID action on prostaglandin synthetase might be important. Further studies are needed to understand the pharmacologic basis for the drug interactions described in this work, and will be of general interest to the study of viral defenses at the mucosal surface.

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