Inflammatory Reactions in Extraoral Tissues in Mice after Intragingival Injection of Lipopolysaccharide

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Intragingival (ig) injection into mice of lipopolysaccharide (LPS) from Prevotella intermedia or Escherichia coli elevated the activity of the histamine-forming enzyme, histidine decarboxylase (HDC), in the mandible, liver, lung, and spleen, with a time course similar to that seen with intravenous (iv) injection. The effect of ig injection was less than that of iv injection but similar to that of intraperitoneal (ip) injection. The ig injection also increased hepatic serotonin, reflecting platelet accumulation. In galactosamine-treated mice, the minimum ig dose of LPS needed to induce lethal hepatitis was very small (less than that needed by ip injection). These results support the idea that the LPS produced in oral tissues may be transported easily to extraoral tissues and, in some cases, may cause inflammatory or immune responses. It also may influence the pathogenesis of some systemic diseases.

In recent years, oral infection has become recognized as a factor affecting the course or pathogenesis of a variety of systemic diseases, such as cardiovascular diseases (endocarditis, atherosclerosis, coronary heart disease, and stroke), diabetic complications, and pneumonia [1–3]. In addition to the metastatic spread of infection from oral tissues, bacterial components derived from oral bacteria, such as endotoxins or lipopolysaccharides (LPS), also could have a role in inflammatory or immune responses in extraoral tissues and influence the pathogenesis of such systemic diseases.

Histamine is a typical mediator of inflammation. This vasoactive amine modulates various immune responses, including the production of cytokines [4–12]. Conversely, several cytokines themselves (and LPS as well) stimulate the production of histamine in various tissues through the induction of the histamine-forming enzyme, histidine decarboxylase (HDC) [13–18].

Serotonin (5-hydroxytryptamine; 5HT) is another important mediator of inflammation, and this vasoactive amine also affects immune reactions, including the production of certain kinds of chemokines [19]. In the blood of mice, 5HT is present in platelets [20, 21]. Human platelets, on the other hand, contain both histamine and 5HT [20, 22]. We have shown elsewhere that a small dose of LPS stimulates platelets to accumulate predominantly in the liver [23–25] and that the LPS-induced changes in 5HT levels in the liver in mice reflect the translocation of platelets to the liver from the circulation [23, 24].

We previously showed in mice that intravenous (iv; via a tail vein) or intraperitoneal (ip) injection of an LPS from Prevotella intermedia, Porphyromonas gingivalis, or Escherichia coli elevates HDC activity in the mandible and in other tissues (liver, lung, spleen, and tibia) [26, 27]. The iv or ip injection of these LPSs also causes platelets to accumulate in the liver [26].

Galactosamine (GalN) sensitizes experimental animals to the lethal effects of LPS. Fulminant hepatitis with severe hepatic congestion occurs after their coinjection, with rapid death ensuing within a few hours [28–30]. Injection of a combination of GalN with heat-killed gram-negative bacteria (instead of LPS), gram-positive bacteria, staphylococcal enterotoxin B, or tumor necrosis factor (TNF) also induces enhanced lethality [31, 32]. Thus, this experimental system with GalN has been used widely as a model of endotoxin shock or sepsis and is useful for detecting the in vivo effects of a low dose of LPS.

On the basis of the background described above, in this study we assessed the in vivo effects of LPS from P. intermedia or E. coli on extraoral tissues after its intragingival (ig) injection into mice. We measured changes in HDC activity in several tissues and changes in 5HT (i.e., platelets) in the liver and examined the lethality of an ig injection of these LPSs in mice treated with GalN.

Materials and Methods

Mice and reagents. Male BALB/c mice (7–8 weeks old) were obtained from our university’s facility for experimental animals. LPS from P. intermedia ATCC 25611 was prepared as described elsewhere [27], and LPS from E. coli O55:B5, prepared by the Boivin method, was obtained from Difco Laboratories. Each LPS was dissolved in sterile saline. D(+)-GalN was purchased from Sigma Chemical. GalN was dissolved in distilled water and, after
the pH of the solution was adjusted to 7.0 with NaOH solution, it was injected ip (0.1 mL/10 g of body weight).

LPS ig injection. Because a 31 G needle (external diameter 0.26 mm, top angle 22°; Hamilton) is flexible, it was encased in autopolymerizing resin before being attached to a microsyringe (Hamilton). A portion of mucobuccal fold near the lower right first molar (M1) of each mouse (not anesthetized) was pierced by the needle almost at a right angle to the alveolar bone; the needle was stopped when it reached the bone. LPS solution (5 μL/mouse) then was injected slowly. In our initial attempt, bleeding or leakage of injectate sometimes occurred. With more experience, this task was completed (by H.F.) without problems.

LPS injected iv and ip. LPS solution was injected at a volume of 0.1 mL per 10 g of body weight.

Assay of HDC activity. Mice were decapitated, and their tissues were removed rapidly before being frozen in a box containing dry ice. The HDC activity in each tissue was assayed and expressed in nanomoles of histamine formed per hour per gram of tissue (nmol/h/g) \([27, 33]\). Measurement of 5HT in the liver. In brief, the liver of each mouse, removed rapidly as described above, was stored in a box containing dry ice until assayed. The 5HT level in the liver was determined within 1 week of its removal. Frozen liver tissue \((0.3 g)\) was homogenized in a tube containing 3 mL of 0.4 M HClO\(_4\), 0.1% cysteine-HCl, and 2 mM EDTA–2Na; the supernatant collected after centrifugation was used for the 5HT assay. After 5HT was separated by column chromatography, it was measured fluorometrically, as described elsewhere \([23]\). The amount of 5HT was expressed in nmol/g of liver tissue.

Data analysis. Experimental values are given as mean ± SD. The statistical significance of differences was analyzed by a Student’s unpaired \(t\) test after analysis of variance. \(P < .05\) was considered to be significant.

Results

Elevation of HDC activity after ig injection of \(P.\) intermedia LPS. After ig injection of 5 μL of LPS solution (500 μg/mL), there was elevated HDC activity in all tissues examined (spleen, lung, and mandible; figure 1). This dose of LPS corresponds to 2.5 μg/mouse \((≈ 100 μg/kg)\). The time course of the change in HDC activity in these tissues was essentially the same as that seen when LPS is injected iv \([27]\). Even though LPS was injected into the gingiva of the lower right jaw, the time course of the HDC response was similar on both the right and left sides of the mandible. There was no significant difference in the magnitude of the response between the 2 sides.

Figure 2 shows the HDC activity induced in the mandible (right and left sides), spleen, liver, and lung after ig injection of various doses of \(P.\) intermedia LPS. In all tissues examined, HDC activity increased in a dose-dependent manner. At 10 μg/mouse \((400 μg/kg)\), the HDC elevation in the mandible was greater on the right side (injected side) than on the left.

We reported elsewhere \([27]\) that a dose-dependent HDC elevation occurs in the mandible and spleen after iv injection of the \(P.\) intermedia LPS used in the present study. The present data (figure 2) show that the HDC activity levels induced in these tissues by ig injection of this LPS at 2.5 μg/mouse \((100 μg/kg)\) are quantitatively similar to levels induced by iv injection at 0.25 μg/mouse \((10 μg/kg)\), as we reported elsewhere.
Figure 2 shows that ig injection of saline itself produced a modest but significant increase in HDC activity in the spleen and liver, especially in the spleen.

**Increase in 5HT in the liver after ig injection of P. intermedia LPS.** As mentioned above, an increase in 5HT in the liver reflects an accumulation of platelets. We showed elsewhere [26] that iv injection of an LPS prepared from *P. intermedia* or *P. gingivalis* induces an elevation of 5HT (i.e., an accumulation of platelets) in the liver. As shown in figure 3, ig injection of *P. intermedia* LPS also increased 5HT in the liver. The effects were significant, even at the lowest dose used, 0.63 μg/mouse (25 μg/kg).

**Lethality of *P. intermedia* LPS in mice treated with GalN.** When *P. intermedia* LPS was given iv, it was lethal in some GalN-treated mice at a dose as low as 0.063–0.125 μg/mouse (2.5–5 μg/kg; figure 4). It was lethal when injected ig at ≥0.63 μg/mouse (≥25 μg/kg). The approximate minimum lethal dose of ig-injected LPS was less than that for ip injection (figure 4). In these experiments, all dead mice had severe congestion in the liver.

**Effects of ig injection of *E. coli* LPS.** An ig injection of *E. coli* LPS also induced an elevation of HDC activity in the liver and lung and an increase in 5HT in the liver. The levels reached were similar to those induced by ip injection (figure 5). By ig injection into GalN-treated mice, *E. coli* LPS induced lethality at doses lower than those needed by ip injection (figure 6). Injection ig caused death with as little as 2 ng/mouse (80 ng/kg).

**Discussion**

In the present study, we found that ig injection of an LPS from *P. intermedia* or *E. coli* elevated HDC activity in a dose-dependent manner in the mandible, liver, lung, and spleen. The time course of the HDC elevation was similar to that previously reported after iv injection of the same LPS [27]. The magnitude of the HDC elevation induced by ig injection of *E. coli* LPS was similar to that induced by ip injection; however, the ig dose needed was ~10 times higher than the iv dose to produce a response of similar magnitude in these tissues. An ig injection of LPS from *P. intermedia* or *E. coli* also increased 5HT (i.e., platelets) in the liver. In GalN-treated mice, the minimum ig dose of LPS needed to induce lethality was very low (e.g., 2 ng of *E. coli* LPS per mouse) and less than that required when the ip route was used.

There are several implications of our findings. The O-antigen of *E. coli* LPS consists of repeating units of oligosaccharide [34]. However, *P. intermedia* LPS lacks such a structure [35]. As reported elsewhere, an iv injection of either LPS induces an elevation of HDC activity and an accumulation of platelets in the liver. The potency of *E. coli* LPS is ≥10 times greater than that of *P. intermedia* LPS [26]. In the present investigation of these responses, the potency of *E. coli* LPS was also greater than that of *P. intermedia* LPS on ig injection, which suggests that the mechanisms by which they induce these reactions may be similar, regardless of the route of administration. Because these reactions are also induced by interleukin (IL)-1 and TNF [13, 24] and because the elevation of serum IL-1 levels induced...
by iv injection of *E. coli* LPS is much greater than that induced by *P. intermedia* LPS [36], it is likely that, in addition to a direct contact between LPS and gingival tissues or extraoral tissues, cytokines including IL-1 and/or TNF (locally or systemically produced) may be involved in the induction of these reactions.

In addition to cardiovascular diseases and diabetic complications (see the introductory section), bacterial LPS has been suggested as being involved in the pathogenesis of disseminated intravascular coagulation [25], nephritis [37], arthritis [38, 39], gastritis [40], cholangitis [41], and hepatitis [42, 43]. Murakami et al. [44] demonstrated that transgenic mice with an anti–red blood cell autoantibody fail to develop anemia when bred in germ-free conditions, whereas they develop the disease when bred in a conventional environment. In addition, when such animals either were transferred from germ-free conditions to conventional conditions or were injected with LPS, they developed anemia. Oral administration of LPS in mice also results in an increase in its level in the blood and in an exacerbation of collagen-induced arthritis [39]. The present results suggest that LPS produced in oral tissues may easily diffuse into the circulation, enabling it to produce systemic effects. These findings lead us to speculate that LPS derived from oral bacteria may affect the pathogenesis of the systemic or extraoral diseases listed above.

The elevation of HDC activity induced by LPS or cytokines leads to enhanced histamine production [14, 45]. HDC is fully induced in various tissues by *E. coli* LPS, even in mast cell–deficient mice [25], and the major cells in which HDC is induced are thought to be vascular endothelial cells and granulocyte precursor cells [46–48]. The newly formed histamine is released from the site of its formation without being stored [45, 49–51]. Elsewhere, we have tentatively suggested that the histamine newly formed as a result of HDC activity should be referred to as “neohistamine,” to distinguish it from mast cell histamine, and we suggested that such neohistamine may play a part in a number of phenomena: for example, in the exacerbation of collagen-induced experimental arthritis [52], in muscle fatigue [33], in the prolonged accumulation of gastric acid induced by LPS and IL-1 [40], and in the symptoms of temporomandibular disorders [53]. Our present results support the idea that neohistamine may also be involved in mediating the systemic inflammatory actions of LPS derived from oral bacteria.

GalN consumes uridine nucleotides in hepatocytes during its metabolism, and this results in a decrease in RNA synthesis [54]. Within 2 days of its injection into rats, this agent induces a type of hepatitis resembling human viral hepatitis [54], and nucleolar fragmentation is an early event after the injection of GalN [55, 56]. As mentioned above, in GalN-treated mice, the lethal effect of LPS is markedly augmented, and fulminant hepatitis with severe hepatic congestion occurs within a few hours. In the present study, we found that ig injection of either LPS examined (from *P. intermedia* or *E. coli*), even at a very low dose (e.g., 2 ng of *E. coli* LPS/mouse), induced fulminant hepatitis with severe congestion. We emphasize that LPSs from some oral bacteria have properties similar to those of *E. coli* LPS [57]. In humans, infection by hepatitis viruses does not necessarily induce hepatitis, and the mechanism underlying the development of the disease is not clear. A highly speculative idea might be that, in humans infected with hepatitis virus (and in GalN-treated animals), the presence of LPS (including that of oral origin) might be sufficient to trigger the development of the disease through the stimulation of inflammatory reactions.

Piguet et al. [58] showed that depletion of platelets by an anti–platelet antibody afforded significant protection against the mor-

![Figure 5](image-url)

**Figure 5.** Comparison of effects of intragingival (ig) and intraperitoneal (ip) injections of *Escherichia coli* lipopolysaccharide (LPS) on histidine decarboxylase (HDC) activity and hepatic 5-hydroxytryptamine (5HT). Mice were given LPS dose and were killed 4 h later. Each value is the mean ± SD of 4 mice. *P < .05, **P < .01, and ***P < .001 vs. control (no injection). *P < .05 and **P < .01, between the 2 groups indicated.

![Figure 6](image-url)

**Figure 6.** Lethality of *Escherichia coli* lipopolysaccharide (LPS) in mice treated with galactosamine (GalN). At 1 h after intraperitoneal (ip) injection of GalN (1000 mg/kg), mice were given indicated dose of LPS by intragingival (ig) or ip injection. Lethality within 12 h of LPS injection was recorded (n = 5).
tality induced by LPS plus GalN. A few years later, we showed that there is a marked accumulation of 5HT (i.e., platelets) in the liver in association with the hepatic congestion underlying such lethality [59]. In the present study, ig injection of LPS also resulted in an increase in 5HT in the liver (reflecting an accumulation of platelets), which suggests a possible link between platelets and hepatic congestion. Of interest, like the LPS-induced elevation of HDC activity described above, the lethal hepatitis induced by LPS plus GalN also is mediated by TNF and/or TNF plus IL-1 [29, 30]. In any case, our current results suggest that LPS from oral bacteria may produce an effect on platelets.

In terms of the induction of HDC activity, the spleen is highly sensitive to LPS [26, 27]. In the present study, we noted that ig injection of saline produced a significant elevation of HDC activity in the spleen, suggesting that even the (unidentified) inflammatory stimulus delivered to gingival tissues by simply giving an ig injection might itself have been enough to cause a slight elevation of HDC activity in the spleen.

As shown in figure 2, the LPS-induced HDC activity was similar on the right and left sides of the mandible at a dose of 100 μg/kg, but much higher on the right side (ig injection side) than on the left side when the dose was 400 μg/kg. Although we have no data to help us explain these results, it is conceivable that there are 2 compartments capable of inducing HDC activity in the mandible, one sensitive and the other less sensitive to LPS. The less-sensitive compartment on the right (injected) side may have been responsible for the additional elevation of HDC activity seen at 400 μg/kg. We speculate that these 2 compartments may be hematopoietic precursor cells in the bone marrow and endothelial cells in the microcirculation, because these are 2 major candidates for the cells in which HDC is induced [48]. However, to test this hypothesis, we need to perform further studies. In conclusion, our results suggest that the LPS produced in oral tissues may diffuse easily into the circulation, where it may be carried to extraoral tissues to induce inflammatory or immune responses and/or to influence the pathogenesis of some systemic diseases.

References


