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COMMENTARY

LEUKOTRIENES AS MEDIATORS OF ISCHEMIA AND SHOCK*

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Leukotrienes (LT) are a group of eicosanoids formed from arachidonic acid metabolism via the lipoxygenase pathway. There are two basic types of leukotrienes: (a) those containing a small sulfido-peptide side chain (e.g. LTC_4, LTD_4 and LTE_4), and (b) those that are non-peptidic (e.g. LTB_4). All originate from the same parent compound (i.e. LTA_4). Although the presence of the leukotrienes has been appreciated since at least 1940 when Kellaway and Trethewie [1] described the Slow Reacting Substance of Anaphylaxis (SRS-A), it was not until recently that SRS-A was related to the eicosanoid family. A few years ago, Samuelsson and coworkers [2] elucidated the chemical structure of SRS-A and coined the term leukotriene as the generic name of these interesting lipid derivatives. We now know that SRS-A consists of a mixture of LTC_4, LTD_4 and LTE_4 and, together with LTB_4, comprises a group of potent naturally occurring substances that have the potential to contribute significantly to the pathogenesis of a variety of inflammatory and ischemic disorders [3].

The purpose of this paper is to review the evidence for the role of leukotrienes as mediators of ischemia and shock-related circulatory disorders. In contrast to other eicosanoids (i.e. prostaglandins, thromboxanes) [4], relatively little is known about the role of leukotrienes in shock. This is partly due to the recent discovery of the chemical nature of leukotrienes, the difficulty of measuring leukotrienes in blood, and the lack of availability of precise pharmacologic modulators of leukotrienes (i.e. leukotriene synthesis inhibitors and leukotriene receptor antagonists). During the last 3 years, however, significant progress has been made to solve these problems.

Biological actions of leukotrienes

The leukotrienes exert a variety of important biological actions in several cell types and, therefore, can contribute to a variety of major pathophysiological effects. Table 1 lists the major biological actions of the four primary naturally occurring leukotrienes (i.e. LTB_4, LTC_4, LTD_4 and LTE_4). Since LTC_4 is converted to LTD_4 and then to LTE_4, there is a prolongation of the biological effects of the peptide leukotrienes as they undergo metabolism. In many systems, LTD_4 is more active than LTC_4 so that, at least initially, metabolism enhances the effects of peptide leukotrienes. However, LTE_4 is usually less active than LTD_4, although it still has appreciable biological activity [5]. Moreover, since LTE_4 accumulates during leukotriene metabolism, LTE_4 may play a more important role in the pathogenesis of shock than was previously thought.

As can be seen in Table 1, both the major peptide and non-peptide leukotrienes exert significant microcirculatory actions, promoting leakage of fluid across the capillary endothelial membrane in virtually all vascular beds studied [6]. LTB_4 has, in addition, potent chemoattractant and chemotactic actions, thus recruiting mobile scavenger cells (e.g. leukocytes, macrophages) to an area already producing LTB_4. LTB_4 then contributes to the adherence of these mobile cells to the endothelial membrane of the vasculature potentially contributing to obstruction of blood flow in these areas. Despite these effects, LTB_4 appears to lack significant stimulatory effects on smooth muscle [7].

In contrast, the peptide leukotrienes stimulate a variety of types of muscle (e.g. vascular smooth muscle, respiratory smooth muscle). LTC_4 and LTD_4 are potent bronchoconstrictors, producing marked contraction of isolated tracheal or lung parenchymal strips at concentrations as low as 10^{-10} to 10^{-9} M [8]. LTC_4 and LTD_4 are also very effective stimulators of vascular smooth muscle. This vasoconstrictor effect has been shown to occur both *in vivo* and *in vitro* in a variety of vascular beds including the pulmonary, coronary, cerebral, renal and mesenteric vasculatures [9–11]. Recent data show that LTC_4 and LTD_4 also constrict human coronary arteries [12]. These vasoconstrictor effects of peptide leukotrienes are more potent than any other known vasoconstrictors except those of thromboxanes and their derivatives [13]. The effectiveness of leukotrienes as constrictors of the vasculature supplying vital organs (i.e. heart, brain, lung) places them as potential ischemia-producing or ischemia-enhancing substances. Clearly, the vasoconstrictor effects of LTC_4 and LTD_4 are of great significance. In fact, leukotrienes can be considered as candidates for mediation of coronary vasospasm.

Since peptide leukotrienes are potent coronary constrictors, they are implicated in a variety of cardiac disorders including arrhythmias, conduction blocks and cardiac depression. It is too early to assess the role of leukotrienes in arrhythmias, but

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Table 1. Biological actions of leukotrienes

Biological action	LTB_4	LTC_4	LTD_4	LTE_4
Chemoattractant and chemotactic actions	+++	0	0	0
Bronchoconstriction	0	+++	+++	±
Vasoconstriction	0	+++	++++	++
Decreased cardiac function	0	Secondary to vasoconstriction		
Enhanced capillary leakage	++	++	++	0

Key: 0 = no effect, ± = possible effect, ++ = moderate effect, +++ = strong effect, ++++ = very strong effect (from Ref. 4).

significant evidence has been accumulated recently that peptide leukotrienes do not exert a direct negative inotropic effect [14–16]. LTC_4 and LTD_4 clearly depress myocardial contractility, but they do so secondarily via marked coronary vasoconstriction. This has been shown in rat, cat, guinea pig and rabbit hearts [16]. In papillary muscles isolated from all four of these mammalian species, LTC_4 and LTD_4 failed to exert any significant inotropic effect at concentrations that markedly constricted the coronary vasculature [16]. Thus, LTC_4 and LTD_4 do not appear to exert direct effects on cardiac muscle, a finding consistent with most of the eicosanoids including the prostaglandin endoperoxides and the thromboxanes [14].

Evidence for leukotrienes as mediators in ischemia and shock

In order for a substance to be considered a true mediator of a pathophysiologic state, it must fulfill four basic criteria. These are: (a) exert one or more pathophysiologic effects, (b) occur in increased amounts endogenously during the development of the disease state, (c) mimic some of the effects of the disease state when administered exogenously, and (d) diminish the disease state by inhibition of the mediator. In the previous section, a number of pathophysiologic effects of leukotrienes were presented. Virtually all of these effects including chemoattraction, increased chemotaxis, bronchoconstriction, vasoconstriction, and capillary leakage contribute to the severity of ischemia and exacerbate both circulatory and anaphylactic shock. Evidence for the other three criteria will now be evaluated, and gaps in our knowledge will be mentioned.

Formation of leukotrienes in ischemia and shock. Leukotrienes are present in very low concentrations normally and even during some conditions that favor their production. Moreover, rapid metabolism and clearance from the circulation render measurement of leukotrienes in circulating blood quite difficult. At present, radioimmunoassay (RIA) techniques have proven usually fruitless in measuring leukotrienes in the circulating blood of animals in shock [17, 18]. Nevertheless, some information has been obtained to suggest the production and accumulation of leukotrienes in shock states. Ogletree *et al.* [19] were the first to show increased lipoxygenase pathway products in shock. Using chromatography and mass spectrometry techniques, they found that the concentrations of 5-hydroxyeicosatetraenoic acid (5-HETE), a precursor of leukotrienes in the lipoxygenase pathway, increased about 4-fold in pulmonary lymph during the time pulmonary vascular permeability occurred in sheep during endotoxemia. Since this significant increase occurred prior to the full development of endotoxic shock and because it correlated with an important pathophysiologic effect to this study, it is of considerable significance. Nevertheless, 5-HETE is only a precursor of leukotrienes and is not itself a leukotriene. Following this study, Luderitz *et al.* [20] found that mouse peritoneal macrophages produced large amounts of LTC_4 in response to incubation of these cells with endotoxin. This leukotriene production was blocked by the lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA). In a recent study, Hagmann and coworkers [21] developed elegant high performance liquid chromatography (HPLC) methods for measuring both LTC_4 and LTD_4 in body fluids of rats in endotoxic shock. They found that over 80% of these peptide leukotrienes are rapidly cleared from the blood into the bile in normal and endotoxic shock rats. These workers also [22] showed that the elevated LTC_4 and LTD_4 concentrations returned to control values within 2 hr. Part of the reason for this rapid return to baseline values is the rapid metabolism of LTC_4 and LTD_4 to N-acetyl derivatives of these leukotrienes and to LTE_4 [23].

These findings accentuate the fact that it is difficult to measure leukotrienes in circulating blood because of the interference with the RIA, the rapid clearance from the blood, and their high metabolic rates. Radioimmunoassays have been successful in non-hemoglobin containing solutions (e.g. Krebs–Henseleit, Ringer's solution) in perfused organs or in isolated tissue preparations. Thus, more innovative techniques like HPLC of regional body fluids will have to be applied to this problem to answer the questions of where and how the leukotrienes are produced during shock.

Pharmacologic blockade of leukotrienes in ischemia and shock. It would be of considerable significance to be able to ameliorate ischemic and shock states employing inhibitors of leukotriene biosynthesis and antagonists of leukotriene receptor mediated action. A number of lipoxygenase inhibitors are available for study in shock [24]. However, there are a number of problems with their use and with interpretation of their effects. First, all known lipoxygenase inhibitors appear to be free-radical scavengers and, therefore, also inhibit the formation of potentially toxic free radicals (e.g. superoxide, hydroxide radicals) also formed during activation of

the lipoxygenase pathway of arachidonic acid metabolism. Second, many lipoxygenase inhibitors when given at high concentrations also inhibit cyclooxygenase so that their effects are difficult to interpret. Third, several of the classical lipoxygenase inhibitors (i.e. diethylcarbamazine, NDGA) have a very short half-life, thus making *in vivo* studies difficult to perform.

One approach to this problem is to employ carefully selected doses of multiple lipoxygenase inhibitors. In one such study, Hock *et al.* [25] employed three different types of lipoxygenase inhibitors in rats subjected to traumatic shock. One inhibitor, CGS-5391B, a dual lipoxygenase and cyclooxygenase inhibitor, protected in this lethal model of shock, significantly improving survival time and curtailing the production of a cardiotoxic peptide, myocardial depressant factor (MDF). Previous studies showed that pure cyclooxygenase inhibitors (e.g. indomethacin, meclofenamate) did not protect in this model of shock. Employing a lipoxygenase inhibitor without cyclooxygenase inhibitory activity, CGS-5677, these investigators found very similar effects to CGS-5391B. This suggests that inhibition of lipoxygenase products is important in traumatic shock. The third inhibitor to be used in this study, piriprost (U-60,257) is primarily a glutathione-*S*-transferase inhibitor blocking the formation of LTC_4, LTD_4 and LTE_4 from LTA_4. Piriprost also protected in traumatic shock to the same degree as the other two lipoxygenase inhibitors, suggesting that the peptide leukotrienes are important in mediating the pathogenesis of traumatic shock. Diethylcarbamazine, another lipoxygenase inhibitor, was also found by two separate groups to protect in lethal endotoxemic shock [26, 27]. More recently, Leprán and Lefer [28] extended these findings to acute myocardial ischemia. Propyl gallate, an effective lipoxygenase inhibitor, prevented the extension of ischemic damage following acute myocardial ischemia in the cat. This cardioprotective action of propyl gallate suggests that lipoxygenase products including leukotrienes may mediate the ischemic process in myocardial ischemia. Thus, the best available evidence suggests, although does not prove, that inhibition of leukotriene synthesis is beneficial in ischemia and shock [29].

In addition to inhibiting leukotriene synthesis, one can employ leukotriene receptor antagonists [30]. Two such agents are currently available. FPL-55712, a short acting agent which inhibits LTC_4 and to a lesser extent LTD_4, and LY-171883, a relatively long acting specific LTD_4 receptor antagonist [30], have been studied in shock states. Hagmann and Keppler [26] first demonstrated that the leukotriene receptor antagonist FPL-55712 protected mice in lethal endotoxic shock. FPL-55712 given every 30 min for 6 hr reduced mortality from 100 to 0%. Cook and coworkers [31] confirmed a protective effect in endotoxic shock using LY-171883 in the rat. LY-171883 moderated the hypotension and the neutropenia resulting from *Salmonella enteritides* lipopolysaccharide. LY-171883 was also found to dose-dependently improve survival time and curtail MDF production in rats during traumatic shock [32]. FPL-55712 has also been shown to reduce the extension of infarct size [33]. Thus, the leukotriene receptor antagonist studies are consistent with the lipoxygenase inhibition studies and point to an important role of leukotrienes in the pathogenesis of ischemic and shock states. However, more work is necessary particularly with longer lasting blocking agents.

Administration of exogenous leukotrienes and their shock-like effects. Administration of exogenous leukotrienes or leukotriene precursors has only been studied by a very few investigators since these substances have not been available other than in very small amounts. In 1981, Smith *et al.* [34] infused soybean lipoxygenase into unanesthetized sheep and found marked pulmonary injury characterized by increased lung lymph flow and an increased microcirculatory permeability similar to that observed in endotoxemia. Although leukotriene concentrations were not measured in the lymph, these effects are nonetheless quite interesting. In 1983, Lux *et al.* [35] reported that synthetic LTC_4 at a dose of 5 μg/kg produced marked hypotension, bradycardia, hypoxia and acidosis in conscious guinea pig. Thus, a shock-like state could be mimicked solely by administration of a leukotriene. Moreover, the hypotension and the bradycardia, but not the acidosis or hypoxia, could be reversed [35] by administration of thyrotropin releasing hormone (TRH). Recently, infusion of small amounts of LTD_4 intracoronarily was found to produce a profound coronary vasoconstriction [11]. Thus, exogenously administered peptide leukotrienes alone can produce significant ischemia or even conditions similar to shock. Controlled dose-related infusions of mixtures of leukotrienes are needed to determine the patterns of these pathophysiologic effects and to compare them to a variety of ischemic and shock states. These studies should be coupled with administration of appropriate leukotriene receptor antagonists to separate direct receptor mediated effects from nonspecific secondary actions of the leukotrienes.

Integration of leukotrienes into the pathogenesis of ischemia and shock

Leukotrienes appear to act in a variety of ways to promote ischemia and shock. Their effects are widespread in a number of vital organs which are affected in shock [36]. Thus, LTC_4 and LTD_4 tend to produce significant reductions in systemic and regional blood flow. LTB_4 aggravates the effects of smooth muscle vasoconstriction by trapping white cells and macrophages in the microcirculation, further reducing blood flow to the lungs, the splanchnic viscera and the kidney. All the leukotrienes promote vascular leakage of fluid in these vital organs. Moreover, vascular and pulmonary tissue can produce significant amounts of peptide leukotrienes [37, 38] and thus can propagate the effects of leukotrienes once they are released from blood cells or other sources. Figure 1 summarizes the consequences of these compound effects. All of these organs (i.e. heart, lungs, kidneys, intestines and pancreas) are major target organs in circulatory shock. In the case of the heart, the LTC_4 and LTD_4-induced coronary constriction compromises cardiac performance further weakening the already compromised circulatory system. In the lungs, LTB_4 attracts

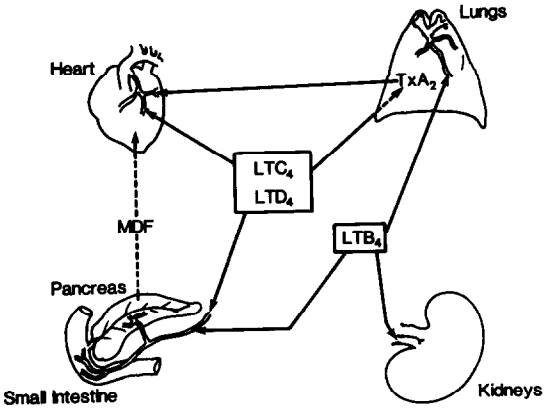

Fig. 1. Scheme of leukotriene-mediated effects in the pathogenesis of shock. Abbreviations: MDF, myocardial depressant factor; and TxA_2, thromboxane A_2.

mobile macrophages capable of releasing several mediators, including thromboxane A_2, which promote platelet aggregation (i.e. thrombosis) and further aggravate already existing vasoconstriction. The peptide leukotrienes promote bronchoconstriction and pulmonary vasoconstriction, thus enhancing the hypoxia of shock. The peptide leukotrienes promote splanchic vasoconstriction along with LTB_4-induced mesenteric microcirculatory impairment due to attraction of macrophages. The resulting conditions favor bowel ischemia and lysosomal disruption (i.e. particularly in the liver and pancreas during shock) leading to the formation of toxic factors including MDF which further depress myocardial performance [39]. Renal function is similarly compromised by the leukotrienes during shock accentuating the sympathetically mediated renal vasoconstriction and tubular necrosis usually observed in ischemia and shock.

Summary and conclusions

Leukotrienes have been implicated as mediators of ischemia and shock. Recent evidence has been obtained supporting the four major criteria of acceptance of leukotrienes as mediators of shock, namely (a) increased concentration in body fluids during shock states, (b) ability to exert significant pathophysiologic effects which aggravate ischemia and shock, (c) amelioration of the shock state by leukotriene synthesis inhibitors and leukotriene receptor antagonists, and (d) production of a shock-like state by exogenous administration of leukotrienes.

In conclusion, both LTB_4 and the peptide leukotrienes (e.g. LTC_4, LTD_4 and LTE_4) also known as the slow reacting substance of anaphylaxis (SRS-A) can be considered as mediators of ischemia and shock. Although difficulties exist with measuring leukotrienes in circulating blood and in obtaining long lasting selective blockers of leukotriene synthesis, innovative experiments measuring leukotrienes in bile and other body fluids and in employing specific leukotriene receptor antagonists have helped in assessing the significance of the leukotrienes in shock states. Additional studies are necessary to evaluate these findings in perspective, and to compare and contrast the role of leukotrienes to that of other vascular mediators including prostaglandins and thromboxanes, as well as non-eicosanoids including serotonin, histamine, angiotensin II and vasopressin, all of which can play a mediator role in ischemia and shock states. Further clarification of these issues promises to open exciting new chapters in shock research.

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