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Interleukin-1, platelet derived growth factor, free radicals and monocyte aryl hydrocarbon hydroxylase activity in liver disease. Role of cell communication

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Abstract—Monocytes were isolated from blood of human origin and cultured in supplemented Leibovitz (L-15) medium for 24 hr. The medium was then decanted and filtered, and all subsequent tests were done on monocyte conditioned medium (MCM). The monocytes of patients with liver disease spontaneously secrete temperature-sensitive arylhydrocarbon hydroxylase (AHH) inhibitory factors detectable in the MCM. Anti-interleukin-1 antibody (IL-1Ab) reduced the AHH inhibitory activity of the MCM, suggesting that part of the AHH inhibitory activity was due to interleukin-1 (IL-1). Platelet derived growth factor did not affect AHH activity. Interleukin-1β was detectable in MCM but did not differ significantly between patients and normal volunteers. A time course experiment indicated that interleukin-1β inhibited hepatocyte AHH activity after only 2 hr of incubation. Catalase partially blocked the AHH inhibitory activity of MCM suggesting that activated oxygen intermediates are partially involved in the AHH inhibitory activity of the MCM. Simultaneous incubation of interleukin-1β and catalase did not prevent or augment the inhibitory action of IL-1 on AHH activity. IL-1 stimulates collagen synthesis and elevates serum procollagen type 3 peptide (P-III-P). Results indicated that serum P-III-P was elevated in blood sources producing temperature-sensitive AHH inhibitory factor.

Arylhydrocarbon hydroxylase (AHH*) activity is mediated by cytochrome P450, is involved in the metabolism of many drugs, xenobiotics and endogenous substrates, and is usually associated with the liver but found in most tissues [1]. The activity of this enzyme is inhibited by compounds that stimulate the reticuloendothelial system [2]. The activity of this enzyme is decreased in patients with cirrhosis [3, 4] and in animal models of liver disease [5, 6]. This study will attempt to determine if interleukin-1 (IL-1), platelet derived growth factor (PDGF) and activated oxygen intermediates are involved in the depression of AHH activity in liver disease.

Materials and Methods

Patients (N = 12) had active chronic liver disease (primary biliary cirrhosis, N = 6, or alcohol-induced liver disease, N = 6) as reflected by histology. Patients had normal WBC counts and monocytes (%) within normal limits and no evidence of infection or systemic inflammation. Serum bilirubin was abnormal in 10 of 12 patients and 7 of the patients consumed alcohol. Normal volunteers (N = 12) were included for comparison.

Murine hepatocytes were isolated as previously described [2]. Peripheral blood monocytes, isolated under aseptic conditions using the method of Peterson [7], yielded 6×10^5 monocytes/10 mL. Endotoxin-free medium (< 0.04 ng/mL) was used for all cell preparations screened for endotoxin using the Limulus amoebocyte lysate assay (Sigma). Macrophages were identified by non-specific esterase staining [8]. Monocyte conditioned medium (MCM) was collected after 24 hr and filtered through 0.22 μm Millex filters; 1 mL of the medium was then incubated with hepatocytes (1.5×10^5 cells) in suspension for 2 hr at 37°. Hepatocytes were collected by centrifugation (50 *g* 1 min) and AHH activity was measured [4]. MCM did not directly neutralize hepatocyte AHH or interfere with its measurement nor did it affect cell viability. IL-1 is sensitive to heating at 70° for 1 hr [9]. Anti-human IL-1β polyclonal antiserum (25 μL) raised against the recombinant molecule (Cistron Biotechnology) was incubated overnight at 4° with 1 mL MCM (25 μL IL-1Ab neutralizes 12.5 half-maximal units of human recombinant IL-1 in the C3H thymocyte proliferation assay). Similar experiments were done using a non-specific antibody (anti-bovine albumin). MCM (1 mL) was also preincubated with 1870 U of catalase

* Abbreviations: AHH, arylhydrocarbon hydroxylase; IL-1, interleukin-1; IL-1Ab, anti-interleukin-1 antibody; 3-OHBP, 3-hydroxybenzo[α]pyrene; PDGF, platelet derived growth factor; P-III-P, procollagen type 3 peptide; TNF, tumor necrosis factor; and MCM, monocyte conditioned medium.

(25 μL) for 1 hr at 25° prior to the addition of hepatocytes. Catalase (1870 U) neutralizes 1.87 mmole of H_2O_2 per min at pH 7.0 at 25°. Platelet derived growth factor (PDGF; Boehringer, Mannheim) (0.1 to 80 ng/mL) and 20 U/mL IL-1β (R&D) in the presence and absence of catalase (1870 U/mL) were incubated with hepatocytes for 2 hr at 37° in a shaking water bath. Recombinant IL-1β (0, 5, 10, 20, 40 U/mL) was also incubated with monocytes for 2 and 24 hr at 37°. AHH activity was determined in cellular homogenates as described previously [7], and expressed in nanomoles 3-hydroxybenzo[α]pyrene (3-OHBP) formed per milligram cellular protein per hour. Cellular protein concentration was assayed as described [10]. IL-1β was quantitated in MCM using an ELISA (R&D Systems). Procollagen III peptide (P-III-P) was measured in serum (100 μL) by radioimmunoassay (Behring) using ^{125}I-procollagen-III-peptide and anti-procollagen-III-peptide serum raised in rabbit. Elevation of serum P-III-P relates to increased biosynthesis of collagen [11] and rises from 6 ng/mL (normal) to 20–50 ng/mL in fibrosis [11].

An unpaired Student's *t*-test was used to compare two variables, and a Student–Newman–Keul's test was used when more than two variables were compared [12].

Results

Monocytes of patients with liver disease had significantly lower monocyte AHH activity (0.158 ± 0.046 nmol 3-OHBP/mg protein/hr, N = 12) compared to that of normal volunteers (0.41 ± 0.053, N = 19) which was within the normal range [13]. The monocytes of patients with liver disease spontaneously secreted factors that significantly decreased hepatocyte AHH activity compared to non-liver disease patients with normal monocyte AHH activity (Fig. 1). The monocytes of the majority of the liver disease patients (92%) secreted inhibitory factors that were heat sensitive, i.e. heating the MCM to 70° for 1 hr significantly reduced the AHH inhibitory activity of the factor (Fig. 1). AHH activity in isolated hepatocytes without addition of MCM was used as another control in all experiments. In previous experiments we had shown that incubation of hepatocytes for 2 hr in a shaking bath at 37° did not alter hepatocyte AHH activity [7]. Incubation of the MCM with IL-1Ab significantly reduced the AHH inhibitory activity of the MCM (Fig. 2). The heat-sensitive AHH inhibitory MCM in 9 of these 11 patients (82%) had reduced AHH inhibitory activity when preincubated with IL-1Ab.

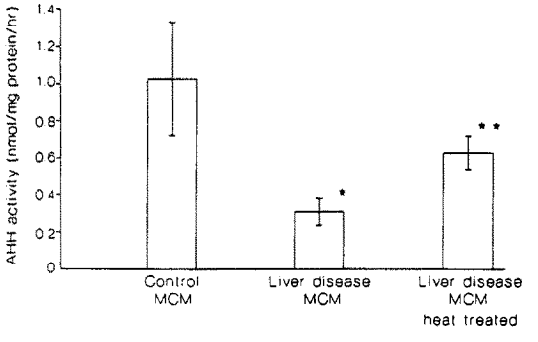

Fig. 1. Secretion of hepatocyte AHH inhibitory factor by monocytes of patients with liver disease compared to normal volunteers and the effect of heat treatment. Control hepatocyte AHH activity was assessed using hepatocytes in the presence of L-15 medium alone to establish normal hepatocyte AHH activity. Each bar represents the mean ± SEM of 11–12 patients or normal volunteers. Key: (*) statistically significant difference compared to controls, $P < 0.05$; and (**) statistically significant difference compared to unheated factor, $P < 0.05$.

Preincubation of MCM with the non-specific anti-sera did not affect the AHH inhibition (data not shown). Preincubation of MCM with catalase significantly reduced the AHH inhibitory activity of the heat sensitive MCM (Fig. 2). The potential role of activated oxygen intermediates in the inhibitory action of the MCM was apparent in 9 of the 11 patients (82%). Heat inactivation of catalase prevented the protective effect observed in the previous experiment. The AHH inhibitory effect of IL-1 in combination with catalase was not different from the effect of IL-1 alone, suggesting that the action of IL-1 on hepatocyte AHH activity is probably not mediated by activated oxygen intermediates but rather that IL-1 and activated oxygen intermediates represent two separate factors involved in depression of AHH activity by MCM (Table 1).

The IL-1β concentration in MCM obtained from monocytes of patients with liver disease was 253 ± 124 pg/mL and not different from normal volunteers (88 ± 39). IL-1β levels were measured before and after treatment at 70°, and results confirmed that heating the MCM to 70° for 1 hr destroys IL-1β activity.

The time course for production of AHH-depressing activity by monocytes suggests that AHH inhibition is obvious 2 hr after incubation of hepatocytes with recombinant IL-1β (data not shown). At shorter incubation times there is no significant inhibition and incubation for longer periods (3 hr) caused greater depression of AHH activity. Incubation of hepatocytes alone for very long periods (6 hr) resulted in reduced AHH activity; 24-hr incubation resulted in a marked loss of AHH activity.

To determine if the AHH-depressing factor being released from monocytes could affect the monocyte AHH activity itself, monocytes were incubated with recombinant IL-1β prior to AHH assay. AHH activity in monocytes that were not exposed to recombinant IL-1β was 0.79 ± 0.2 nmol 3-OHBP/mg protein/hr (data not shown). Incubation of monocytes with 10 or 40 U/mL IL-1 β for 24 hr did not decrease monocyte AHH activity significantly. No effect of recombinant IL-1β on monocyte AHH activity was observed with shorter incubation periods. Hepatocytes were also incubated with increasing concentrations of PDGF (0.1 to 80 ng/mL) and PDGF did not affect hepatocyte AHH activity (data not shown). Serum procollagen-III-peptide was elevated significantly ($P < 0.001$) in patients with liver disease (23.5 ± 2.87 ng/mL, N = 12) compared to normal volunteers (10.1 ± 0.88 ng/mL, N = 12).

Discussion

Activation of Kupffer cells results in secretion of soluble, heat-sensitive factors that decrease hepatocyte and Kupffer cell AHH activity [2, 14, 15]. IL-1 inhibits hepatocyte AHH

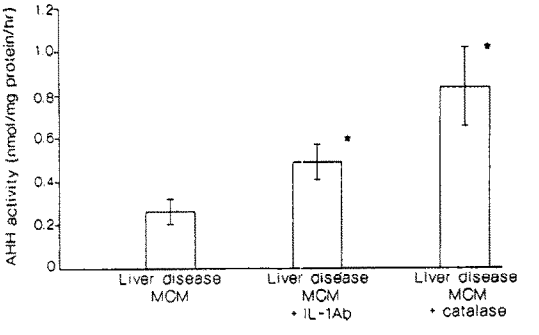

Fig. 2. Effect of IL-1 antibody and catalase on monocyte factor. Each bar represents the mean ± SEM of 11 patients. Key: (*) statistically significant difference compared to untreated factor, $P < 0.05$.

Table 1. Effect of catalase on AHH inhibition due to recombinant IL-1β

IL-1β (U/mL)	Hepatocyte AHH activity (nmol/mg protein/hr)		
	Control	+ Catalase	+ Heat-inactivated catalase
0	0.19 ± 0.0	—	—
10	0.14 ± 0.04	0.12 ± 0.00	—
20	0.10 ± 0.00*	0.15 ± 0.03	0.11 ± 0.01
40	0.10 ± 0.02*	0.09 ± 0.01	—

All samples were replicates using the same hepatocyte preparation. Hepatocytes 1.5×10^5 were incubated with IL-1β (+/− catalase 1870 U/mL) for 2 hr at 37° in a shaking water bath. Values are means ± SEM, N = 4.

* Significantly different from 0 IL-1β, $P < 0.05$.

activity and decreases cytochrome P450 content [15–18]. Kupffer cells are activated in liver disease [19], and consequently secrete many factors including IL-1. Decreased hepatocyte and Kupffer cell AHH activity has been reported in a pig model of liver disease [6]. The decrease in monocyte AHH activity in the pig model [6] and in liver disease patients [4] may occur as a result of monocytes circulating through the liver and becoming activated in response to these factors. Monocytes secrete a temperature-sensitive factor that inhibits hepatocyte AHH activity and preincubation of pig MCM with superoxide dismutase reduces the AHH inhibitory activity [6]. Patients with liver disease have reduced monocyte AHH activity and their monocytes secreted hepatocyte AHH inhibitory factor. MCM from patients with liver disease which inhibited murine AHH activity and could be reversed by addition of IL-1β antibody was found to contain detectable levels of IL-1β but were not different from normal volunteers. These results suggest that the IL-1β level alone is not a good index of AHH inhibitory activity. Addition of IL-β antibody indicated that the inhibition of AHH activity by monocyte conditioned medium is due to IL-1β. The mechanism for the depression of AHH activity by IL-1β remains to be elucidated. These results also suggest that the effect of IL-1 on cytochrome P450 activities may not be species specific as has been described for the effect of interferon on P450 because all of the experiments were conducted using murine hepatocytes to screen human MCM. IL-1β is probably not the only factor involved because heat treatment eliminated IL-1β from the MCM but did not totally restore AHH activity to control levels. IL-1 is fibrogenic, stimulates collagen synthesis [20], and elevates serum P-III-P, a cleavage product in collagen biosynthesis [21]. Serum P-III-P was elevated significantly in liver disease patients whose monocytes secreted AHH inhibitory factor and who had decreased levels of monocyte AHH activity. IL-1 induces PDGF [22], but PDGF did not affect hepatocyte AHH activity. Tumor necrosis factor (TNF) has been reported to depress cytochrome P450-mediated enzyme activity but the action of TNF is an indirect action on the Kupffer cells of the liver, possibly via increased synthesis and release of IL-1 from the Kupffer cells [23]. The role of other cytokines remains to be elucidated.

Catalase partially reversed AHH inhibition, suggesting that activated oxygen intermediates are involved in the decrease in AHH activity due to MCM. Activated oxygen intermediates may be involved in hepatocellular injury [24], and stimulation of collagen production by liver cells [25]. Inhibition of AHH activity by recombinant IL-1β is probably not mediated by activated oxygen intermediates and thus there appear to be two separate mechanisms involved in the decrease of AHH activity by MCM. Neither IL-1Ab nor catalase alone completely prevented the inhibitory activity though maximal concentrations were used, suggesting that other macrophage factors in addition to IL-1 and activated oxygen intermediates are likely to be involved in the decrease in AHH activity observed in patients with liver disease.

Drugs that have been beneficial in the treatment of liver disease have been suggested to inhibit IL-1 receptor binding [26], reduce levels of IL-1 [27, 28] and inhibit synthesis of IL-1 [29]. The inhibitory action of endogenous cytokines on cytochrome P450-mediated AHH activity in patients with liver disease suggests a role for cytokines in the down-regulation of cytochromes P450 reported in an animal model [30] and the depression of related drug-metabolizing enzyme activity observed in patients with liver disease [3, 4].

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