Effect of Peptide Vilon on the Content of Transforming Growth Factor-β and Permeability of Microvessels during Experimental Chronic Renal Failure

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We studied the effect of Vilon in rats 2, 4, and 6 months after the onset of chronic renal failure. Subcutaneous injection of Vilon significantly decreased serum concentration of transforming growth factor-β1 and permeability of mesenteric microvessels in rats 2 months after the onset of chronic renal failure. Our results indicate that the preparation produces a potent homeostatic effect in the early period of chronic renal failure.

Key Words: *Vilon; TGF-β1; permeability; chronic renal failure*

Chronic renal failure (CRF) is a clinical syndrome related to irreversible and progressive damage to the kidney [4]. Microcirculatory disturbances determined by functional activity of the endothelium play an important role in the pathogenesis of this syndrome [10, 13,14]. Much recent attention was paid to immune mechanisms of damage to endotheliocytes during CRF. Cytokines and growth factors are believed to play a major role in this process [7,8,13]. Transforming growth factor-β1 (TGF-β1), an important endogenous bioregulator, produces a potent inhibitory effect on immune reactions [6] and plays a role in reparative processes [9,11]. TGF-β1 has a complex origin; the role of individual cells in its production is not determined. Previous studies showed that TGF-β1 is produced by megakaryocytes and released from platelet granules. TGF-β1 is also secreted by monocytes, fibroblasts, and smooth muscle cells of the vascular wall [6,11]. Endotheliocytes and vascular smooth muscle cells carry receptors for TGF-β1 [12].

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Experimental studies would allow us to use immunomodulatory preparations in clinical practice. Vilon (Lys-Glu) is one of the most promising preparations synthesized at the St. Petersburg Institute of Bioregulation and Gerontology. Previous experiments showed that the preparation has immunomodulatory and thymomimetic properties and modulates inflammation, regeneration, and apoptosis [2,5].

Here we studied the effect of Vilon on serum TGF-β1 concentration and permeability of the endothelium in mesenteric microvessels of rats 2, 4, and 6 months after the onset of CRF.

MATERIALS AND METHODS

Experiments were performed on 105 male outbred rats weighing 150-250 g and obtained from the Rappolovo nursery (Russian Academy of Medical Sciences). CRF was modeled by extirpation of the left kidney and electrocoagulation of 25% right kidney cortex. The development of CRF in rats was confirmed by changes in serum urea concentration and systolic blood pressure (measurement by the blood method). The animals were killed 2, 4, and 6 months after CRF modeling. Morphological study showed that this treatment produces autoimmune glomerulonephritis eventuating in

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CRF [1]. The control group included intact animals not receiving the preparation.

Vilon in a dose of 100 μg/kg was subcutaneously injected to CRF rats using a disposable syringe. Group 2 animals with CRF received placebo. We performed 2 courses of treatment. Course I started on day 6 after surgery; the preparation was administered daily for 10 days. Course II started 20 days after completion of course I and lasted 10 days. Researcher remained uninformed about treatment solution and marking of animals (blind study).

During interpretation of the results it is necessary to take into account that Vilon was administered only in the initial period of CRF; then, no injections were made.

Serum TGF-β1 concentration was measured by enzyme immunoassay according to manufacturer's instruction. We used test systems for quantitative analysis of TGF-β1 in the serum and plasma from humans and rats (DRG Instruments, GmbH).

TGF-β1 concentration in a sample was measured on a PC-compatible DRG Eliza-MAT 3000 photometer (DRG Instruments, GmbH) at 450 nm. Optical density was expressed in units of TGF-β1 concentration using appropriate software.

Functional state of the endothelium in microvessels of experimental animals was estimated by permeability for sodium fluorescein (complex method of vital biomicroscopy) [3].

Functional properties of microvessels were studied using a microscopic video device, which included a MT-9 microscope (LOMO), CCD video camera (ISTA Ltd.), SLV-X55ME videotape recorder (Sony), and KV-2185MT television set (Sony).

Functional state of the endothelium was studied in venules with a diameter of 20-25 μ. The system included small color filters to measure vascular permeability. The transmitted light was transferred to the reflected light. A fluorescent substance was injected intravenously. We performed videotape recording of changes in fluorescence for a specified region. Video materials were processed using a software-hardware package, which included a videotape recorder, IBM-compatible personal computer equipped with AV Master analog-digital converter (Fast Multimedia AG), and Fast Cap 2.5.0 (FAST Multimedia Inc.) and VideoTest 5.0 softwares (ISTA Ltd.). This approach allowed performing a geometric, rapid, and quantitative frame-by-frame analysis of video images.

The permeability coefficient (*P*) serves as a major criterion for vascular permeability. This coefficient reflects the amount of substances crossing a specified part (area) in the vascular wall. The fluorochrome sodium fluorescein with a molecular weight of 376 Da served as an indicator of permeability in microvessels. This substance was administered in a dose of 2.5 mg/kg. The indicator was dissolved in physiological saline immediately before the experiment and injected intravenously in a total volume of 0.2 ml.

The permeability coefficient was estimated from changes in fluorescence and time by the following equation:

$$P=0.25\times dIi(t)\times D\times[Ii(0)/Iv(0)]/[Iv(t)\text{-}Ii(t)\times dt],$$

where *P* is the permeability coefficient (cm/sec), *D* is diameter of the microvessel, *dt* is time interval (sec), *dIi(t)* is the rise of fluorochrome fluorescence in the interstitial space (brightness units/sec), *Iv(t)* is fluorochrome fluorescence in the vessel (brightness units), *Ii(t)* is fluorochrome fluorescence in the interstitial space (brightness units), *Iv(0)* is the average initial intravascular brightness in transmitted light (brightness units), and *Ii(0)* is the average initial extravascular brightness in transmitted light (brightness units). Recalculation was performed using MS Excel 2002 software.

The results were analyzed using Excel 2002 and Statistica 6 software.

RESULTS

Two months after the onset of CRF, TGF-β1 concentration in rats of the placebo group was much higher than in control animals (121.7±2.8 and 106.2±3.5 ng/ml, respectively, $p<0.05$). TGF-β1 concentration reached maximum 4 months after the onset of CRF (158.9±8.7 ng/ml, $p<0.05$), then this parameter slightly decreased by the 6th month, but still surpassed the control level (136.8±10.3 ng/ml, $p<0.05$).

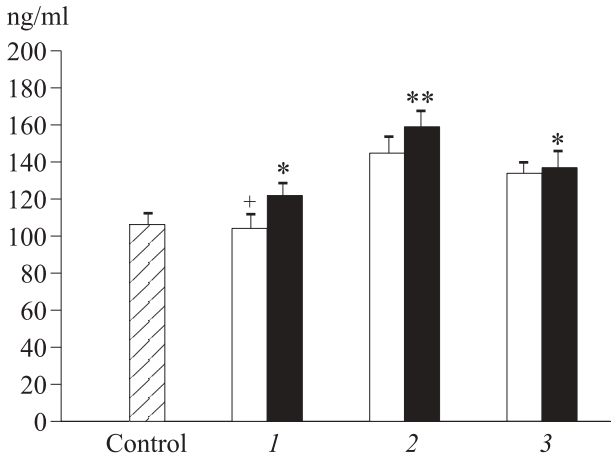

Fig. 1. Serum TGF-β1 concentration in rats at various stages of chronic renal failure (CRF). *$p<0.05$ and **$p<0.001$ compared to the control; $^{+}p<0.05$ compared to placebo. Here and in Fig. 2: CRF, 2 months (*1*); CRF, 4 months (*2*); CRF, 6 months (*3*). Light bars: preparation. Dark bars: placebo.

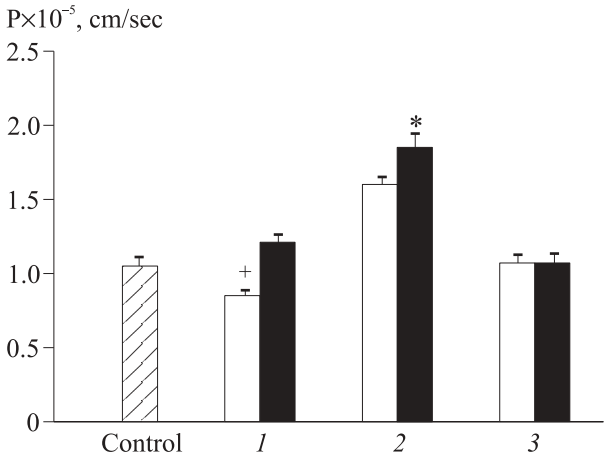

Fig. 2. Permeability coefficient for mesenteric microvessels in rats at various stages of CRF. $p<0.05$: *compared to the control; +compared to physiological saline.

Vilon significantly decreased serum TGF-β1 concentration only 2 months after CRF modeling. In this period serum TGF-β1 concentration was below the control level (104.2±5.1 ng/ml, Fig. 1). TGF-β1 concentration in animals receiving Vilon slightly decreased 6 and, especially, 4 months after the onset of CRF.

Permeability of mesenteric vessels for sodium fluorescein tended to increase in rats of the placebo group 2 months after the onset of CRF ($1.21\pm 0.23\times10^{-5}$ *vs.* $1.03\pm0.17\times10^{-5}$ cm/sec in the control, $p>0.05$). This parameter significantly increased and exceeded the control level after 4 months ($1.85\pm0.31\times10^{-5}$ cm/sec, $p<0.05$). However, by the 6th month permeability of the endothelium was below the control ($1.07\pm0.29\times10^{-5}$ cm/sec). Two months after the onset of CRF permeability of the endothelium significantly decreased in rats of the Vilon group and was lower compared to placebo-treated and control animals ($0.85\pm0.06\times10^{-5}$ cm/sec, $p<0.05$). Vilon tended to decrease the permeability of the endothelium by the 4th month of CRF ($1.60\pm0.27\times10^{-5}$ cm/sec, $p>0.05$). Six months after the onset of CRF, endothelium permeability in Vilon-treated and control rats was similar (Fig. 2).

Serum TGF-β1 concentration in rats of the placebo group increased 2 and, particularly, 4 months after the onset of CRF. These results reflect early and progressive activation of TGF-β1 production under conditions of this CRF model. The absence of this increase on month 6 of CRF can be explained by the development of compensatory changes.

Changes in endothelium permeability were preceded by variations in TGF-β1 concentration. Taking into account low molecular weight of sodium fluorescein (376 Da), we can hypothesize that disturbances in endothelium permeability under these conditions occur at the level of interendothelial gaps and are related to changes in the structure or charge of glycoproteins and decrease in their expression. The development of endothelial injury under the influence of urea, creatinine, and other metabolites, whose concentration in the blood progressively increases during CRF, is followed by stimulation of TGF-β1 receptor expression on the surface of endotheliocytes [15]. Vilon significantly decreased serum TGF-β1 concentration and permeability of microvessels in rats 2 months after the onset of CRF. These data suggest that the preparation has a strong homeostatic effect, which is particularly pronounced in the early period of CRF.

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