Effect of a Hexosylceramide Fraction of the Hemodialysate Solcoseryl on Wound-Healing Angiogenesis

JUHA NIINIKOSKI, M.D., MATTI LAATO, M.D., ROLAND TSCHANNEK, PH.D., AND WOLFGANG FRAEFEL, PH.D.

Departments of Surgery and Medical Chemistry, University of Turku, Turku, Finland, and Institute of Biochemistry, University of Fribourg, Fribourg, Switzerland

Submitted for publication August 30, 1984

Rabbit ear chambers were used to investigate the effects of a locally applied hexosylceramide fraction (Hex-Cer) of the hemodialysate Solcoseryl on wound-healing angiogenesis. The transparent methacrylate ear chambers were inserted under full aseptic precautions. Immediately after implantation the chambers in control rabbits were filled with physiological saline while the chambers in experimental rabbits were injected with a solution containing either 0.3 or 2.5 µg/ml of Hex-Cer, respectively. On the second postoperative day the chambers were reinjected with the corresponding solutions. Thereafter the chambers were examined three times weekly. The onset and rate of the neovascular response were measured by using a standard dissecting microscope equipped with a camera. Vessel growth in ear chambers treated with Hex-Cer at the lower concentration showed very little difference from that seen in control chambers; in both groups the first appearance of new capillaries occurred on an average of 18 days after implantation, and the chambers became fully filled with vessels by the 30th day. However, in chambers treated with Hex-Cer at the higher concentration, the first invasion of capillaries was detected 3 days earlier than in controls (P < 0.05). Correspondingly, in the chambers treated with Hex-Cer complete vascularization was achieved 7 days earlier than in the control chambers (P < 0.01). It is concluded that locally applied Hex-Cer exerted an accelerating effect on wound-healing angiogenesis.

INTRODUCTION

Reports of several research groups have indicated that a deproteinized extract of calf blood, Solcoseryl (Solco Basle Ltd., Switzerland), has a stimulatory effect on tissue repair. Solcoseryl has been shown to enhance wound blood vessel growth [1], stimulate proliferation of fibroblasts and accumulation of collagen [11, 13], accelerate oxidative energy metabolism [15], and improve viability of tissue in which circulation has been compromised [5, 9, 17].

Among experimental wound models available, the rabbit ear chamber provides a unique method for direct vision, photography, and quantification of a monolayer of capillaries filling a defined experimental wound [6, 16]. In the present work rabbit ear chambers were used to investigate the effects of a locally applied hexosylceramide fraction (Hex-Cer) of Solcoseryl on wound-healing angiogenesis. The reason for this fraction being chosen for the study was that earlier tests on fibroblast proliferation and wound healing indicated that a major part of Solcoseryl's activity is present in the Hex-Cer fraction [3].

MATERIAL AND METHODS

New Zealand White rabbits weighing 2.5–3.5 kg were used. The transparent methacrylate ear chambers were constructed as described by Silver [16]. The animals were anesthetized with intravenously injected fentanyl citrate and fluanisone. The dorsal and ventral surfaces of both ears were shaved and remaining hair was removed with a depilatory. Both ears were disinfected with 70% ethanol and povidone iodine. The ear chambers were inserted under full aseptic precautions. A central sharp punch was used to cut a hole through the ear. A flap was then formed between the dorsal skin and perichondrium by blunt dissection. The ear chamber was inserted through the hole in the cartilage to the dorsal surface.
of the ear and the chamber skirt was placed between the skin flap and the perichondrium. The dorsal skin was trimmed and fixed to the rim of the chamber by means of a ligature (Fig. 1).

Immediately after implantation the ear chambers were treated in a blind fashion. The solutions used were supplied in color-coded ampuls and the color code was not broken until the experiments had been completed. The chambers of control rabbits were filled with physiological saline while the chambers of two experimental groups were injected with a solution containing either 0.3 or 2.5 μg/ml of Hex-Cer, respectively. These solutions as well as the control solution were supplied by the Research Department of Solco Basle Ltd. The Solcoseryl fraction tested contains predominantly hexosylceramides and is obtained by successive Sephadex G25 chromatography, high-performance liquid chromatography, and Biogel P2 chromatography of the hemodialysate. Total acid hydrolysis of this fraction and subsequent analysis of the fragments showed the fraction to be uniform with regard to sugar, this being exclusively β-D-glucose. The ceramide part is not uniform, however; C18 sphingosine and a saturated C16 fatty acid account for 60%, but C20 sphingosine and saturated and unsaturated fatty acids of the C18 series can also be demonstrated in the hydrolysate. Attempts are now being made to synthesize the main component of the fraction, D-erythro-1-(β-D-glucopyranosyloxy)-3-hydroxy-2-palmitoylamino-4-trans-octadecene.

On the second postimplantation day the chambers were reinjected with the corresponding solutions. For this purpose the spring clip and the coverslip above the chamber table were removed and the chamber was refilled, whereafter a new sterile coverslip and spring clip were placed in position.

Ears were examined three times weekly after chamber implantation. Any ear chambers which became infected after implantation were not used for experimentation.

The onset and rate of the neovascular response were measured by using a standard dissecting microscope with a transmitted light source, equipped with a camera. Photographs were taken at appropriate intervals after implantation until the termination of the experiment. The rate of vessel growth was determined by mapping the percentage of total chamber surface area occupied by capillaries as described by Knighton and co-workers [6].

RESULTS

In this series of 30 ear chambers (10 per group) the first new vessels at the edge of the chamber table were seen between the 12th and the 24th days. Before capillary sprouts were visible, the chamber table was covered by exudate containing fibrin, erythrocytes, and leukocytes. Within a few days following the first invasion of capillaries, more vessels appeared on the chamber table at other points around its edge, extending farther each day and converging toward the center. As the zone of new capillaries advanced across the table, those more peripheral vessels which were a few days older continuously changed in pattern and be-
came distinguishable as arteries or veins. Eventually, the vessels from different sides met and anastomosed, and the chamber table was completely covered by a plexus of functioning blood vessels.

As shown in Fig. 2, vessel growth in ear chambers treated with Hex-Cer at the lower concentration showed very little difference from that seen in control chambers; in both groups the first appearance of new capillaries occurred on an average of 18 days after implantation, and the chambers became fully filled with vessels by the 30th day. However, in chambers treated with Hex-Cer at the higher concentration, the first invasion of capillaries was detected on an average of 3 days earlier than in controls \((P < 0.05; \text{analysis of variance})\). Correspondingly, in the chambers treated with Hex-Cer complete vascularization was achieved 7 days earlier than in the control chambers \((P < 0.01)\).

After the first capillaries had entered the chambers the vessel growth was significantly faster in the group treated with Hex-Cer at the higher concentration than in the other groups (Fig. 3).

**DISCUSSION**

As mentioned above, Solcoseryl is a protein-free extract of calf blood with various components. Each milliliter of this nonantigenic solution contains 45 mg of dry substance of which approximately 75% consists of inorganic salts. The remainder contains amino acids, hydroxy- and ketoacids, deoxyribose, purines, acid and alkaline polypeptides, and glycolipids.

In the study of Pichotka and co-workers [12] Solcoseryl trebled the oxygen consumption by homogenized guinea pig liver. This observation prompted attempts to determine whether it would enhance the oxygen consumption by tissues in vivo and be effective in the treatment of nonhealing ulcers.

In animal experiments, Solcoseryl accelerated the healing of gastric ulcers in rats and of dermal wounds in guinea pigs, rabbits, and pigs [1, 8, 19]. Epinephrine and ergotamine-induced gangrene of rats' tails could be prevented with Solcoseryl [5]. Similarly, treatment with Solcoseryl improved the survival of skin grafts and healing of donor sites in rats [9]. In experimental burns, intravenous Solcoseryl therapy resulted in a significant reduction of the healing time [14]. The effects of Solcoseryl were attributed to accelerated ingrowth of blood vessels, enhanced proliferation of fibroblasts, and augmented oxidative energy metabolism [4, 13, 15, 17]. In 1979,
Niinikoski and Renvall [11] tested the effect of Solcoseryl on developing granulation tissue in rats. Subcutaneous sponge implants were used as an inductive matrix for the growth of granulation tissue. Chemical analyses of the implants demonstrated a stimulatory effect of Solcoseryl on vascularization, cell proliferation, and collagen formation in the repair tissue. Clinical studies have suggested that Solcoseryl, when administered locally or systemically, is effective in the treatment of patients with a variety of chronic and intractable ulcers [2, 7].

In the present blinded study a hexosylceramide fraction of Solcoseryl stimulated blood vessel growth in rabbit ear chambers; the first capillary sprouts became visible earlier than in control chambers and the daily vessel growth rate was also enhanced.

The exact mechanism of action of Hex-Cer can only be speculated upon at the present time. It has been assumed that the chemical structure of Hex-Cer with its lipophilic and hydrophilic parts is such that it may become attached to plasma membranes. This is in agreement with the finding that Solcoseryl treatment can maintain the respiration of fibroblast cultures damaged by irradiation [10], and that Solcoseryl improves the viability of tissues suffering from ischemia [2, 5, 9, 17]. In addition, Solcoseryl has been shown to enhance penetration of capillaries into trophic lesions by increasing the fibrinolytic activity around the developing blood vessels [18]. Whatever the mechanism of action, the effects of Solcoseryl and the Hex-Cer fraction tested are similar. Both treatments enhance the invasion of blood vessels in the reparative tissue, this being decisive for the subsequent course of the healing process.

REFERENCES