A Modified Rabbit Ear Chamber and an Example of Its Application for Intravital-Microscopic Study on Acute Effects of Topical Thermal Stimulation

Makishige ASANO, Chiyoji OHKUBO and Akihiko SASAKI
Department of Physiological Hygiene, the Institute of Public Health, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan
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Abstract—A pre-existing plastic rabbit ear chamber was modified to be applicable for intravital-microscopic observations in pharmacological as well as pathophysiological studies on inflammation. The chamber consists of a base disk, a mica (or glass) cover-slip and a holder ring. The base disk has a round-table with a central protrusion in which a heat conductor of platinum wire is so implanted near the protrusion that thermal stimulation can be given to the regenerated tissue between the table and the coverslip.

The authors have previously modified (1) the rabbit ear chamber (REC) described by Ahern et al. (2) in the suitable dimensions for Japanese domestic rabbits and have utilized it for intravital-microscopic studies on pharmacological (3–7) as well as physiological (1, 8–12) events of the microcirculation. Recently, they further modified the REC to be used for observing microcirculatory effects of a topical thermal stimulation to the living tissue in it, according to Allison et al. (13–15).

This REC is a transparent round-table chamber consisting of three parts as before: a base disk having an observing round-table and three pillars for supporting the chamber, a mica (or glass) cover-slip for the chamber, and a holder ring. Its basic design and dimensions are the same as the former one (1) as shown in Fig. 1-a, b. The disk and the ring are produced by the die-casting of acrylic resin. The round-table with a diameter of 6.4 mm and a height of 1.15 mm has a central protrusion with a diameter of 0.7 mm and a height of 50 μm to keep a tissue space for regenerating vasculature.

The modification is that a heat conductor of platinum wire with a diameter of 0.5 mm and a length of 7 mm was implanted in the round-table through a hole. The point of the platinum wire is positioned about 1 mm apart from the central protrusion on the same plane with the surface of the round-table and the platinum wire is extended to the outside of the disk and bent at a right angle to prevent inward displacement, as illustrated in Fig. 1-b-3. The hole for the heat conductor is pierced with a precision mini drilling machine (Chibi-I, Itoya, Tokyo) and the external part of the heat conductor of platinum wire is appropriately fixed with a small amount of quick self-curing acrylic resin (Unifast, GC Dental Industrial Corp., Tokyo) on the reverse side of the base disk of REC, as seen in Fig. 1-a.

The REC is installed in an ear lobe of the adult animal through four holes perforated at one time with a specially devised steel punch (1, 2). The central one of these holes serves for the round-table and the outer three holes serve for the supporting pillars of the disk. For successful regeneration of tissue within the REC, it is prerequisite to remove the skin from both sides of the ear cartilage in a circular area inscribing all the outer three holes. Blood vessels must be left on the cartilage by using an artificially blunted surgical knife and small forceps. In addition, the central hole is punched near the end of the ear and adjacently to an arch of the central auricular artery ramifying in two directions. The observed tissue with ingrowing microvasculature between the cover-slip and the round-table is studied in order to follow its complete process.
of maturation (about 6 weeks after the operation).

The conscious animal is placed in a standard rabbit box (metal drum), and the ear lobe with the REC is fixed gently on the observing stage of a microscope. Then microcirculatory system within the REC is observed directly and/or on a TV monitor via a VTR circuit (11). This procedure is fundamental for observing the basic state of the...
microcirculation within the REC, regardless of whether the apparatus is equipped with the heat conductor, and can be repeated for a long period (more than several months), unless the REC is damaged by factors such as infection, ischemia and external forces.

Here is an example of the application of this REC for studying the microcirculatory changes induced by a thermal stimulation of 60°C for a period of 2 min. The heat was given by contacting a nichrome wire, which had been previously warmed to a fixed temperature, to the platinum wire. Principal features of the microcirculatory events observed with vital microscopy before, during and following the heat stimulation were as follows:

Under normal and basal conditions, the microcirculation within the REC showed spontaneous rhythmic changes consisting of both increase and decrease in the diameter of vessels as well as the volume and velocity of blood flow, suggesting the existence of vasomotion (1, 3–7, 10, 11). Within 1 min after starting the heat stimulation, an intense constriction began to develop on arterioles surrounding the point of the platinum wire (Fig. 2-b). The vasoconstriction developed sometimes strikingly in a localized manner, so that the constricting arterioles looked like pinched-in bowels (Fig. 2-b, c). Following withdrawal of the heat stimulation, arterioles quickly recovered from the constriction, although they remained in the sausage-like partial contraction more or less (Fig. 2-d, e, f). On venules surrounding the point of the platinum wire, a slight dilatation developed,
while no constriction occurred during the heat stimulation (Fig. 2-b, c). However, in venules, thrombus formations and stases developed during and following the heat stimulation (Fig. 2-e, f). Capillary dilatations, hemorrhages and stases also developed during the heat stimulation around the point of the platinum wire. After withdrawal of the heat stimulation, intravascular streaming leukocytes increased in number at a given site in the vessels, and some of them adhered to the lumens, especially in the postcapillary regions, singly or in groups. No vasomotion occurred at all throughout the observation during and even 30 min following the heat stimulation.

Of course, the REC can be applied for further observations on acute and chronic microcirculatory effects of the thermal injuries even at higher temperatures and for longer durations and for observations on pharmacological treatments for inflammation due to the thermal stimulation. The findings of such experiments will appear in detail elsewhere in the near future.

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