CONCENTRATION AND TURNOVER OF INTRAPERITONEAL HYALURONAN DURING INFLAMMATION

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Abstract—Aseptic peritonitis was induced in rabbits by intraperitoneal injection of irritating agents, mainly starch suspensions. The inflammatory response was followed in the peritoneal lavage fluid by cell counts (average increase about 800-fold the first day) and hyaluronan concentration (average increase about 200-fold on the second and third days). The turnover rate of hyaluronan was studied by injecting tritium-labeled hyaluronan intraperitoneally and by following the appearance of tritiated water in serum. In control animals given trace amounts of hyaluronan, half-lives of 1–14 h were recorded. When the labeled polysaccharide had been mixed with 10 mg/ml of unlabeled hyaluronan, the half-life was approximately one day. Rabbits with ongoing peritonitis exhibited half-lives between 1 and 16 h. It was concluded that there was a large individual variation in uptake kinetics, that the removal process could be receptor mediated, and that the increase in intraperitoneal hyaluronan in peritonitis mainly was due to an increased production of the polysaccharide rather than a decreased rate of removal.

INTRODUCTION

Hyaluronan, an important component of the extracellular matrix, is a high-molecular-weight linear polysaccharide built from alternating residues of N-acetyl glucosamine and glucuronic acid (1). Hyaluronan is synthesized at the cell
surface by a membrane-bound enzyme, and the synthesis proceeds by extrusion of the polymer into the pericellular space (2). Accumulation of hyaluronan has been observed in tissues and organs during inflammatory conditions, and it is known that various inflammatory mediators such as interleukin-1, growth factors, and prostaglandins (3) can stimulate hyaluronan synthesis in fibroblasts.

The catabolism of hyaluronan has been studied in some detail in recent years. Part of the polysaccharide is taken up and degraded locally, e.g., in the skin (4). Another part is removed from the tissues by lymph (5, 6) to be degraded in lymph nodes (7). Some enters the general circulation and is taken up by the hepatic endothelial cells and degraded (8, 9).

In the present study we have investigated whether an inflammatory response in the peritoneal cavity causes an increase in hyaluronan in peritoneal fluid and if so, whether the increase can be ascribed to an increased production or an impaired catabolism of the polysaccharide. Aseptic peritonitis was induced by intraperitoneal injections mainly of starch suspensions (10). The hyaluronan concentration was measured in peritoneal lavage fluid up to nine days after the injections by the use of a specific radioassay (11). The turnover rate of the polysaccharide was determined by a recently developed technique using $^3$H-labeled hyaluronan (12).

**MATERIALS AND METHODS**

*Animals.* Nineteen New Zealand White rabbits, male and female, with an average weight of 2.9 kg, were used in experiments where animals were injected intraperitoneally with irritating agents to induce peritonitis and followed by peritoneal lavage. One rabbit died within 24 h after the injection, but the remaining animals showed no signs of distress. An additional five control rabbits, male and female, with an average weight of 2.9 kg, were lavaged without previous injection.

To study the elimination rate of hyaluronan from the peritoneal cavity, another 14 New Zealand White rabbits, ten males with average weight of 2.3 kg and four females with average weight of 2.9 kg, were used. Peritonitis was induced in four of the males. The female rabbits were, as all of the rabbits, caged separately and thus in the preovulatory phase of the estrous cycle (13). Furthermore, the progesterone contents of serum samples at the time of the intraperitoneal injections were at very low levels (14), indicating that the female rabbits had not ovulated. There were no signs of distress in these animals.

All rabbits in this study had the same living conditions with food and water ad libitum and separate cages. All animal experiments were approved by the Uppsala Regional Animal Ethics Committee.

*Injectates.* Dulbecco’s phosphate-buffered saline (NaCl 8.0 g/liter, KCl 0.2 g/liter, Na$_2$HPO$_4$ $\times$ 12H$_2$O 2.9 g/liter, and KH$_2$PO$_4$ 0.2 g/liter) was used in preparing most solutions and will henceforth be referred to as PBS.

Suspensions or solutions of 0.1% latex beads (styrene divinylbenzene copolymer latex. Dow Chemical Company, Midland, Michigan), 3% thioglycolate (Oxoid, Basingstoke, Great Britain), and 2% starch (Kebo, Stockholm, Sweden) in PBS were injected intraperitoneally to induce aseptic peritonitis.