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DIFFERENT TISSUE REACTIONS TO ACTIVATED CARBON FIVERS – PATHOLOGICAL AND IMMUNOLOGICAL FINDINGS AFTER SUBCUTANEOUS IMPLANTATION –

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We have shown here effects on biological affinity of activated carbon fivers (ACFs) comparing with that of asbestos. ACFs and asbestos have been implanted into subcutaneous tissues of mice for 6 months. Histological and immunological investigations have been performed. As results, tissue samples implanted asbestos showed a severe inflammatory reaction and formation of abscess-like mass in their implanted tissues. Changes of CD_4^+/CD_8^+ were markedly occurred in the asbestos implanted group. Between ACFs, A-20 showed better tissue affinity histologically and immunologically in indicating that a micro-pore size with a surface of those composted materials may be important as a factor of biological responses to those materials.

Keywords: activated carbon fivers; mice; tissue reaction; histology; CD4; CD8; inflammation

Recently, new materials based on carbon composites materials have been dramatically focused on their applications covered with wide fields, even in biological and/or medical fields. However, tissue reactions to ACFs have not been clarified. Those investigations may become important biologically and/or ecologically when these materials have been applied widely.

In this paper, different repairs of murine subcutaneous tissues were examined after the implantation of activated carbon fibers (ACFs). Additionally, time course changes in peripheral T lymphocytes were investigated. These materials of A-10, A-15 and A-20 have been provided by Osaka Gas Co. LTD (Japan, Figure 1).

Thirty animals were anesthetized by intraperitoneal injection of Nembutal (30 mg/Kg). Then, fragmented ACFs stirred with physiological

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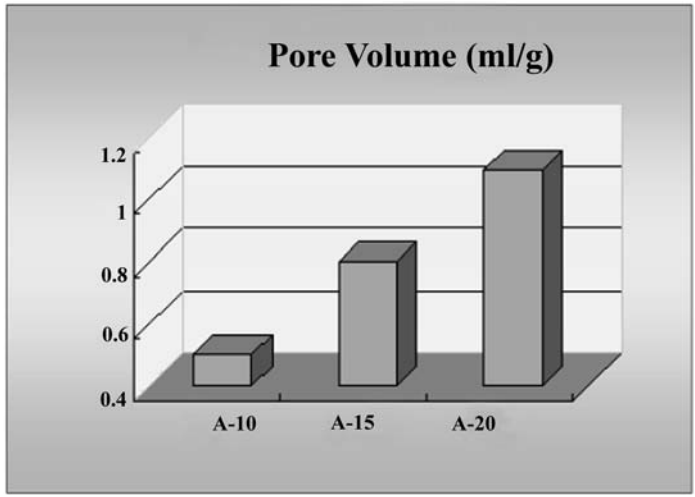

FIGURE 1 Figure shows a comparison of pore volume of each ACFs.

saline were implanted into the subcutaneous tissue of the back. Each group (Control, A-10, A-15, A-20 and asbestos group) consisted on six animals. After complete recovery from anesthesia, animals were freely moved in the cage and fed for six months. Peripheral blood was sampled from the tail vein in each animal at one, 2, 3, 6 months after the implantation. Antibodies of CD_4 and CD_8 (Pharmingen, USA) were used for sorting T lymphocytes by a flow cytometer (FACS Clibur, Becton Dickinson, USA). For histological examinations, tissue samples were removed as a mass after animals were sacrificed under the deep anesthesia and 1%KCl solution. Those samples were fixed with 10% formardehyde and embedded in paraffin. The thin section of each sample was stained with hematoxyline and eosin.

Figure 2 shows histological aspects of subcutaneous tissue in each group at six months after the implantation. Group of asbestos showed a loss of normal tissue arrangement with formation of abscess and appearance of giant cells resulting from severe inflammation. In A-10 group, the implanted fivers did not covered with fibrous connective tissues. Invasion of inflammatory cells and giant cells were not observed. Those histological aspects were found in A-15 group, however, a newly formed connective tissue seemed to be more tightly covering over the fivers. Interest aspects were found in A-20 group. The fivers were fixed into the subcutaneous tissue tightly covered with fibrous connective tissue without cell infiltration. No giant cells were found. A loose space around the fivers did not observed indicating that when A-series of ACSs used here is applied *in vivo* such as artificial devices, A-20 may be better tissue compatibility *in vivo* than A-10 or A-15.

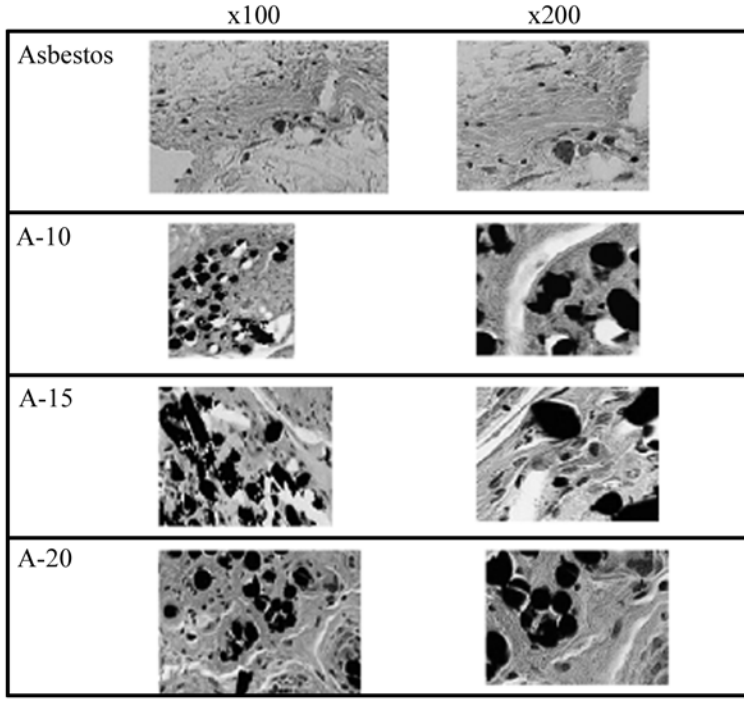

FIGURE 2 Figures show histological aspects of implanted fibers into the subcutaneous tissue.

In the above experimental groups of the implantation model of ACFs, CD_4 and CD_8 of peripheral lymphocytes as T cell makers of immuno-reaction were examined on time passed of 1, 2, 3 and 6 months. Time course changes in ratio of CD_4^+ and CD_8^+ were illustrated in Figure 3. This ratio indicates one of indicators of immuno-responses. Time course of changes in control shows a biological response to ageing used in this experiments. A-10 group showed a S-shaped curve of T lymphocytes differentiation. However, groups of A-15, A-20 and asbestos occurred a wave pattern with an initial declined phase (one month), increased peak (2–3 months) following to a secondary fall (6 months).

An initial and secondary decrease of CD_4^+/CD_8^+ were greater than in the group of asbestos. Degrees of fall of CD_4^+/CD_8^+ were followed: Asbestos > A-15 and A-20 $\geqq$ A $\geq$ 10. An increased peak was appeared in the group of asbestos earlier than other groups at 2 months after the implantation. A similar increase in this phase was appeared in the group of A-15 at 3 months after the implantation. Degrees of increased peak of

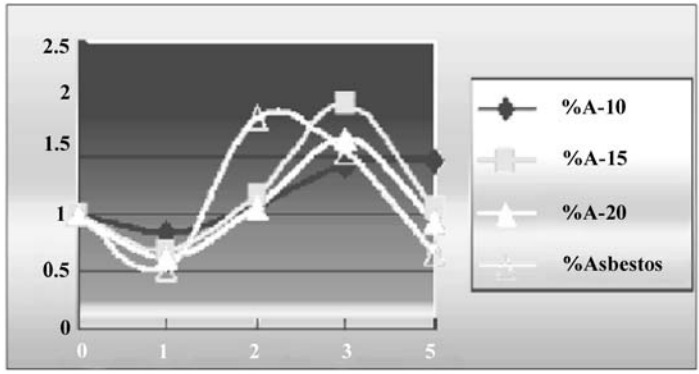

FIGURE 3 Figure shows a time course change in peripheral CD_4^+/CD_8^+ after the implantation of fivers.

CD_4^+/CD_8^+ were followed: Asbestos = A-15 ≧ A-10 = A-20. To simplify the above time course of CD_4^+/CD_8^+, Figure 4 shows a graph comparing with changes of CD_4^+/CD_8^+ on each blood sampled timing of 1, 2, 3 and 6 months after the implantation. Asbestos showed a marked deviation of

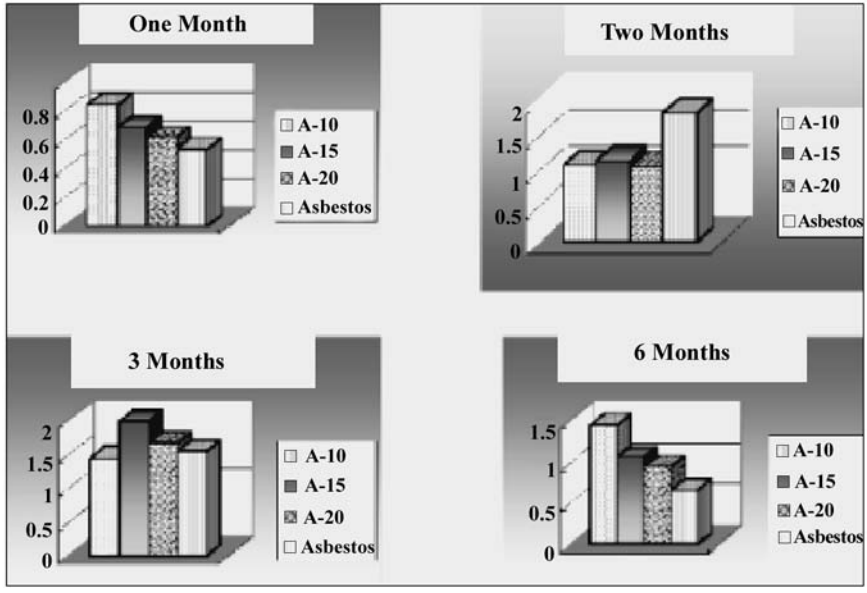

FIGURE 4 Figure shows a comparison of changes in CD_4^+/CD_8^+ ratio between each fiber at different period after the implantation.

CD_4^+/CD_8^+ during this experimental investigation. Within 2 months, in this group, severe inflammatory reaction occurred resulting in a typical histological aspect as shown in Figure 2. Comparing with asbestos, this initial inflammatory reaction occurred at an earlier period (1 month) in the A-10 group and at later period (3 months) in the A-15 group. On time passed, this homeostatic mechanism of T cells was reversed to decrease without recovery to the normal state. On 6 months, an inflammatory reaction in the A-10 group has been still activated and immunological defense mechanism has been altered in the asbestos group (1). However, a more stable reaction of CD_4^+/CD_8^+ was appeared in the A-20 group during the experiment.

Those findings are identical to the histological investigation demonstrated here. Those suggested that some different physical properties of ACFs even in similarly originated materials such as A series may result in a different biological reactions *in vivo*. One of those different properties may depend on a different pore-size of surface between ACFs used here. This possibility will be reported in this journal (2).

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