

Beneficial Effects of Cocoa in Perivascular Mato Cells of Cerebral Arterioles in SHR-SP (Izm) Rats

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Summary As previously reported, the cerebral arterioles are surrounded by unique perivascular Mato cells. They contain many inclusion bodies rich in hydrolytic enzymes, and have strong uptake capacity. They are thus considered scavenger cells of vascular and neural tissues in steady-state. In this study, employing hypertensive SHR-SP (Izm) rats, the viability of Mato cells was investigated. In hypertensive rats, the capacity for uptake of horse radish peroxidase (HRP) and the activity of acid phosphatase (ACPase) of Mato cells were markedly reduced, and on electron-microscopic examination Mato cells were found to include heterogeneous contents and appeared electron-dense and degenerated. Vascular cells exhibited some signs of pathology. However, in hypertensive rats fed chow containing 0.25% cocoa, the uptake capacity and ACPase activity of Mato cells for HRP were enhanced, and on electron-microscopic examination Mato cells appeared healthy, with mitochondria with nearly normal profiles. Signs of pathology in vascular cells were also decreased. Superoxides may impair Mato cells and vascular cells.

Key Words: hypertension, SHR-SP (Izm) rat, cocoa, Mato cell, superoxide

Introduction

Our previous studies [1–5] showed that the unique perivascular Mato cells (fluorescent granular perithelial cells) surrounding cerebral arterioles contained many auto-

fluorescent lysosomal inclusions rich in hydrolytic enzymes, and that they thus appear to be novel cerebral scavenger cells in steady-state.

They are derived from leptomeningeal cells, and exhibit strong capacity for uptake of endogenous and exogenous materials in the cerebral vessels and neural tissue just after birth, but are transformed into foamy cells and degenerate in aged rats. They are provided with epitopes of macrophage lineage such as ED1, ED2, HLA-DR, and scavenger receptor for oxidized LDL [4–7]. Then, Thomas [8] and Williams

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et al. [9] regarded Mato cells as immuno-regulatory cells in the central nervous system. It has also recently been established that Mato cells are the key cells in fibrosis of the cerebral arterioles [8].

As demonstrated previously [9], Mato cells exhibited degeneration in Wistar rats fed vitamin E-deficient chow, though this was protected against by administration of Vitamin E [10]. These findings suggested that viability of Mato cells was associated with the generation of superoxide in the vascular system and cerebral tissue, and that vitamin E reduced oxidative stress.

Recent research on hypertension has shown that angiotensin II enhances the generation of superoxides [11–13].

In this study, to extend findings on the viability of Mato cells under conditions of hypertension, we employed hypertensive rats (SHR-SP(Izm) rats) and studied alterations of function and the ultrastructure of Mato cells and vascular cells, and aimed to prevent the deterioration of Mato cells by oral ingestion of chow containing cocoa-polyphenols.

Material and Methods

This investigation was performed according to the Guide of the Animal Ethics Committee for the Care and Use of Laboratory Animals of Saitama Medical University, Japan.

Fourteen male SHR-SP(Izm) rats at 4 weeks of age were purchased from SLC, Shizuoka, Japan. They were housed in cages maintained in a humidity- and temperature-controlled room.

Systolic blood pressure was measured every week by the standard tail-cuff method. The blood pressure of SHR-SP(Izm) rats (fed SP chow (SLC)) increased rapidly to about 210 mmHg at 7 weeks, and ranged from 250 to 270 mmHg at 14 weeks of age. Most died of cerebral bleeding within 20 weeks of birth. Therefore, for this study, SHR-SP (Izm) rats were sacrificed at 14 weeks of age.

In this study, SHR-SP (Izm) rats 5 weeks of age were divided into two groups. The rats of the first group (NF rats) were fed SP chow for 8 weeks, while the rats of the second group (CF rats) were fed SP chow containing 0.25% cocoa polyphenols for the same period (8 weeks).

For examination by light microscopy, 4 rats 13 weeks of age (NF rats 2, CF rats 2) were perfused transcardially with 10% formalin buffered with 0.1 M phosphate buffer under nembutal anesthesia, and decapitated. Their brains were excised and cut in frontal sections (at 1.5 mm thickness) parallel to the plane containing the lateral and medial thalamic nuclei, and fixed again with 10% buffered formalin for 48 h at room temperature. The procedure for preparation of paraffin sections was performed with routine techniques. Paraffin sections 4 μ m in thickness were deparaffinized and stained with periodic acid Schiff (PAS) and hematoxylin, since the lysosomal inclusions in Mato cells in Wistar rats

are clearly stainable with PAS, as reported previously.

To examine the capacity for uptake of HRP by Mato cells, 2 NF rats and 2 CF rats were infused with 0.3 ml physiological saline containing 20 mg HRP (Type II, Sigma-Aldrich, St. Louis) into a jugular vein under nembutal anesthesia. After 3 h, they were perfused with 100 ml of cold physiological saline also under nembutal anesthesia. Just after this, they were decapitated and their brains were excised. After thorough removal of meningeal tissue, the cortex of the temporo-parietal region was separated and stretched on a glass slide. The stretched specimens were fixed with paraformaldehyde gas at 40°C for 3 min after drying at room temperature. The procedure was performed as described in a previous report [2]. After staining of HRP deposits with diaminobenzidine (DAB) solution, the specimens were observed light-microscopically. To obtain objective data, digital photographs were taken and converted to quantitative data using Image J image analysis software (National Institutes of Health, Bethesda). All data were entered into a MicrosoftTM Excel spreadsheet, and subsequent analysis was performed using Prism 4 (GraphPad Software). Comparisons of optical density between 100 Mato cells of CF and NF rats were performed using the independent sample *t* test. Next, to survey ACPase activity in Mato cells, 10 stretched specimens each of NF and CF rats were stained histochemically with the ACP stain kit (Muto Kagaku, Tokyo, Japan). Furthermore, in order to detect the epitopes (ED2) of Mato cells, the stretched specimens were treated with ED2 antibody (Serotec, Oxford, England) as previously reported [4–7].

For electron-microscopic observation, 3 NF rats and 3 CF rats were anesthetized with nembutal and perfused with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde solutions intracardially. Just after this, their brains were excised and cut in frontal sections at about 1 mm in thickness in the same fashion as for preparation of paraffin specimens. The cerebral cortex of the parieto-temporal region (1 mm \times 1 mm) was separated and postfixed with 1% osmic solution buffered with 0.1 M phosphate solution for several hours at 4°C. After dehydration in a series of ethanols, it was embedded in Quetol 812. The blocks were sliced with an ultramicrotome, and thin sections were stained with uranyl-acetate and lead hydroxide. Specimens were observed with a Hitachi 7000 electron microscope.

Result

The systolic blood pressure of NF rats was 250–270 mmHg at 14 weeks after birth, and that of CF rats almost equal to this. Thus, oral intake of 0.25% cocoa-containing chow for 5 weeks had little effect on blood pressure.

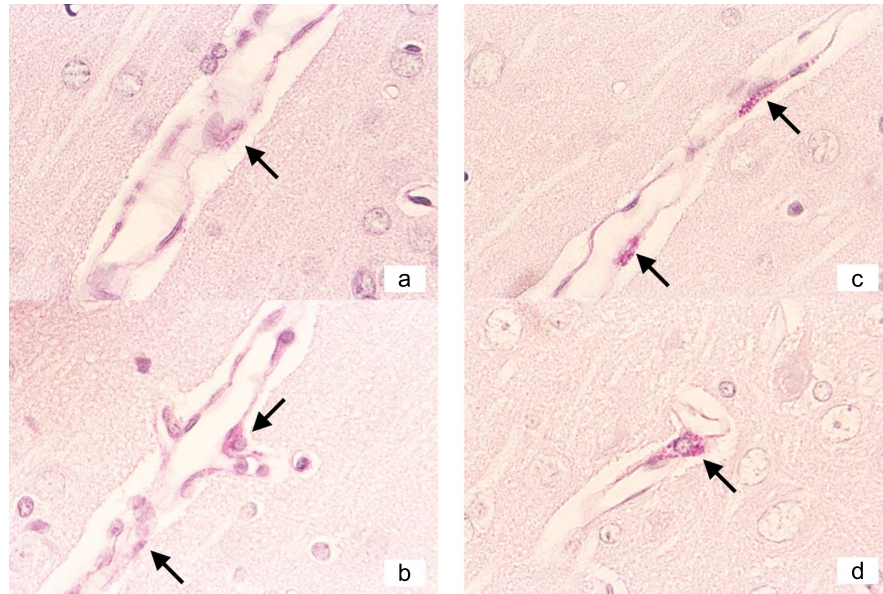


Fig. 1. Fig. 1 shows the light micrographs of Mato cells and cerebral arterioles stained with PAS-hematoxylin method ($\times 400$). a and b are selected from the specimen of SHR-SP (IZM) rats fed with SP chow (NF rats), and c and d from SHR-SP (IZM) rats fed with SP chow containing cocoa-polyphenol (CF rats). a and b (NF rat). Mato cells (arrows) in these figures take irregular shapes, and in their cytoplasm, PAS positive granules are small in number. c and d (CF rats). These figures illustrate the profiles of Mato cells (arrows), which contain a lot of PAS positive granules.

Light-microscopic examination

For comparing the general characteristics and contents of Mato cells in NF rats with those in CF rats, 50 paraffin sections of cerebral cortex stained with PAS-hematoxylin were observed under a light microscope. In the specimens of NF rats, as demonstrated in Fig. 1a and b, the Mato cells along cerebral arterioles appeared atrophic and vacuolized, and their PAS-positive intracellular lysosomal inclusions varied in size and number. In general, the inclusions were stained weakly, and the contours of Mato cells were occasionally obscure. A color photograph (Fig. 1) shows representative findings for Mato cells in this group.

However, as depicted in Fig. 1c and d, Mato cells of CF rats appeared somewhat expanded, and lysosomal inclusions were clearly stained with PAS and hematoxylin. Next, to examine the uptake capacity of Mato cells, a principal marker of the functional activity of Mato cells, stretched specimens of cerebral cortex of NF and CF rats were observed after injection of HRP. As shown in Fig. 2a (NF) and Fig. 2b (CF), the uptake capacity of Mato cells in NF rats was conspicuously decreased, compared with that in CF rats. HRP deposits were fewer in number and finer in NF rats. Occasionally, no deposits were recognized in Mato cells. However, as shown in Fig. 2b, Mato cells had definite profiles, and deposition of HRP was clearly increased in CF rats, reflecting the high uptake capacity of Mato cells in CF rats.

In order to objectively determine the difference in capacity for uptake of HRP between NF and CF rats, the intensity of deposits (optical density) in Mato cells was measured by a photo-densitometric method as described in Materials and Methods. Capacity for uptake by Mato cells in CF rats was moderately increased compared with that in NF rats, as shown in Fig. 5.

In order to examine the lysosomal function, the histochemical technique for demonstration of ACPase was applied to Mato cells in the stretched specimens of NF and CF rats. Reaction product was more evident in CF rats (Fig. 3). In complementary fashion, one marker epitope, ED2, was also checked using stretched specimens in NF and CF rats. The intensity of reaction products in NF rats was clearly weaker than in CF rats (Figures not shown).

The findings noted above indicated that the Mato cells in NF rats had altered features and loss of uptake capacity and ACPase activity, while Mato cells in CF rats were more active than those in NF rats. Ingestion of cocoa thus ameliorated the toxic effects of hypertension in NF rats.

In order to clarify the ultrastructural differences of Mato cells and vascular cells between NF and CF rats, electron-microscopic observation was carried out.

Electron-microscopic observations

a) *Mato cells in NF rats.* As demonstrated in Fig. 4A, C, E, the perivascular Mato cells were situated between the

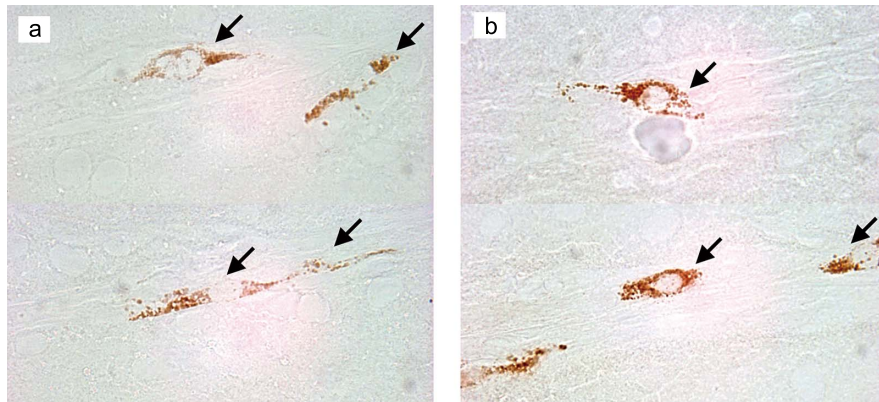


Fig. 2. Fig. 2 shows the uptake of HRP by Mato cells in the stretch specimens of cerebral cortex. Pale oval areas in the cells indicate nuclei ($\times 400$).
 a: NF rat. HRP is roughly distributed through the cytoplasm and extensions.
 b: CF rat. HRP gathers compactly in the cytoplasm, especially around the pale nucleus.

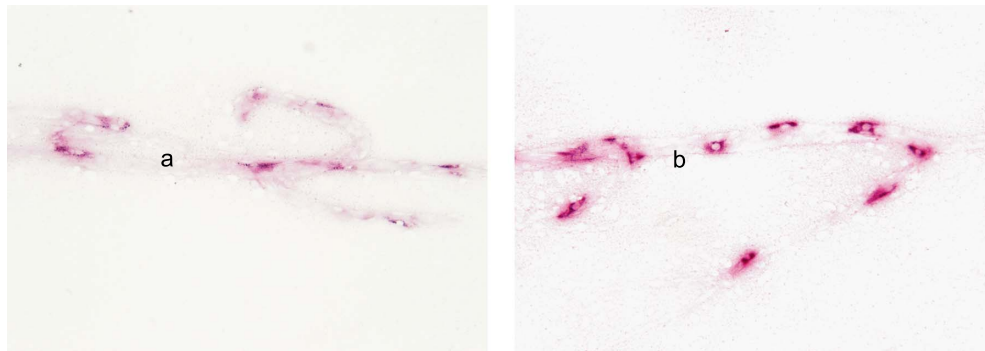


Fig. 3. Fig. 3 shows the enzyme histochemical stain of Mato cells and cerebral arterioles treated with Acid phosphatase staining ($\times 400$).
 a: Acid phosphatase in Mato cells of NF rats.
 b: Acid phosphatase in Mato cells of CF rats.

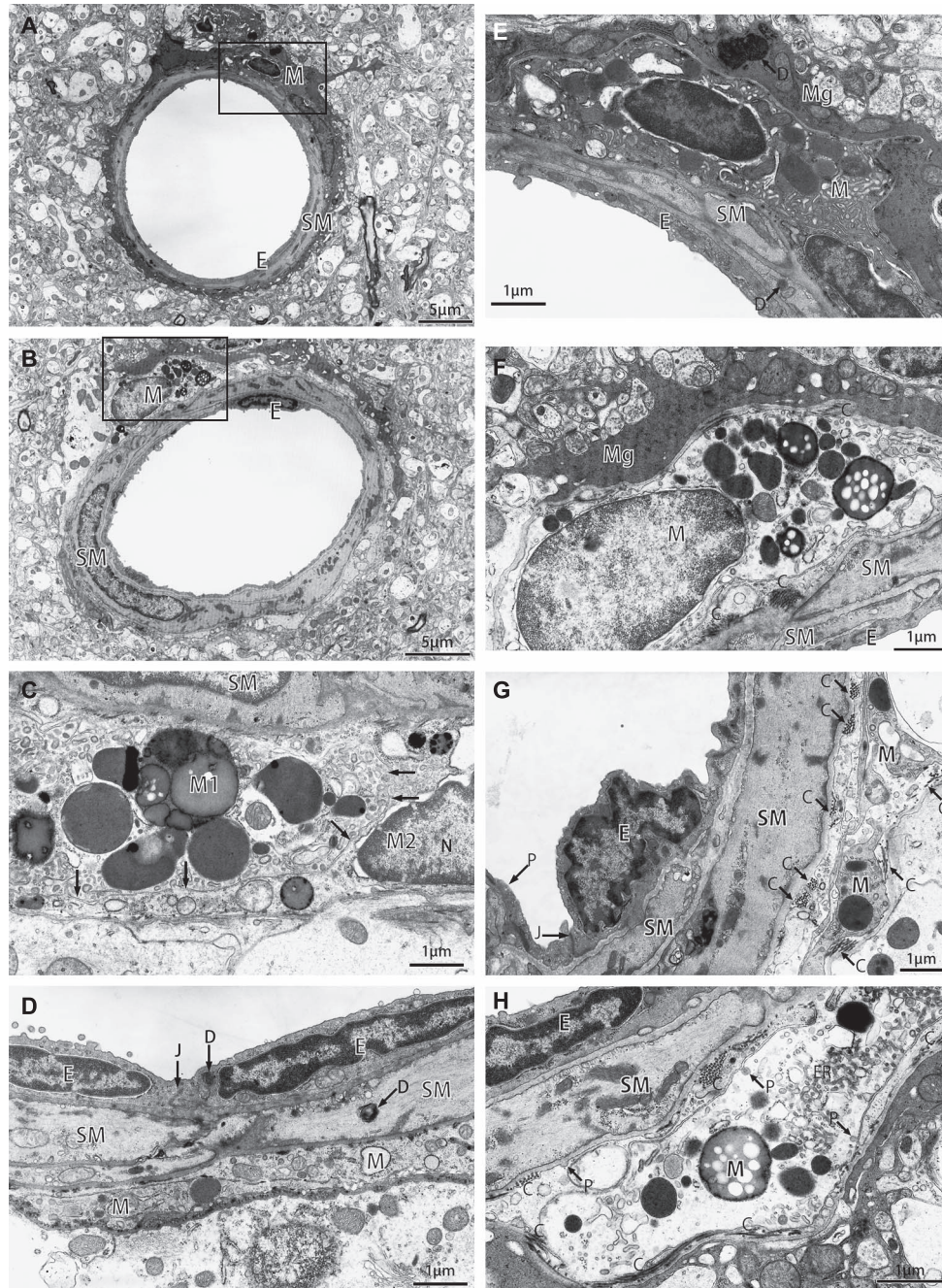
vascular wall and astrocytes or microglia (Fig. 4A, E). They were slender or cuboidal in shape, and contained oval or irregularly-shaped nuclei. However, the electron-density of Mato cells in NF rats varied from pale to dark. Therefore, 100 Mato cells were examined electron-microscopically and classified into three types, based on the intensity of the cytosol and properties of cytoplasmic organelles.

The first type of Mato cells (type I) contained an oval nucleus and several lysosomal inclusions of various shapes and sizes (Fig. 4C M1). Lysosomal inclusions were frequently heterogeneous in content. Their cytoplasm appeared cloudy or relatively pale, and featured numerous vesicles. The endoplasmic reticula and mitochondria exhibited nearly normal profiles. Occasionally, Mato cells of this type were in contact with other types of Mato cells (second or third type; type II and type III), and small numbers of collagen fibers were scattered about them.

The first type of Mato cells accounted for about 25% of

the Mato cells observed in this study. However, in the second type of Mato cell, the cytoplasm and cytoplasmic extensions were semitransparent and appeared cloudy (Fig. 4C M2 and Fig. 4D). They contained irregularly-shaped nuclei, and their perinuclear spaces were occasionally widened. Most of the endoplasmic reticula were expanded, and mitochondria were swollen and their cristae had been destroyed (Fig. 4C, D). Lysosomal inclusions were sparse and smaller in size, and pinocytotic vesicles were scarcely observed. The second type of Mato cells accounted for about 40% of the Mato cells observed in this study.

The third type of Mato cell accounted for 35% of Mato cells observed in this study. The intensity of their cytoplasm was very high (Fig. 4A, E). Cytoplasmic organelles, such as the mitochondria and lysosomal inclusions, were completely dark, and discrimination of individual organelles was difficult (Fig. 4E). As illustrated in Fig. 4E, vesicles, sacs, and vacuoles of irregular shapes, and infoldings of



cytoplasmic membranes were observed in the cytoplasm. Nuclei were pyknotic and markedly homogeneous.

These dark Mato cells were occasionally present in the perivascular spaces between the vascular wall and microglia, and in contact with Mato cells of the second type. Some collagen fibers appeared (Fig. 4A, E) around intense Mato cells (Fig. 4C). These dark Mato cells appeared to have degenerated completely.

A schematic presentation of Mato cells and percentages of Mato cells of each type are shown in Fig. 6.

b) Vascular cells in NF rats. Endothelial cells and smooth

muscle cells exhibited some pathological features (Fig. 4D, E). As the profiles and contents of vascular cells did not differ markedly from those in Mato cells, the descriptions concerning vascular cells in NF rats can be grouped together as follows. In general, endothelial cells were thin and somewhat electron-dense (Fig. 4E). They possessed elongated or irregularly-shaped nuclei and small numbers of dense bodies (Fig. 4D). Their luminal surfaces were smooth, and pinocytotic vesicles were occasionally observed. Sometimes, mitochondria were swollen, and a certain number of vesicles and small dense bodies were distributed through the

Fig. 4. A to I are electron micrographs of Mato cells and cerebral arterioles. A (NF rat) and B (CF rat) are general view of cerebral arterioles, and at the outside of arterioles, Mato cells are displayed (M). The square of A is magnified in E, and that of B is in F. C to E are selected from NF rats, and F to H from CF rats. C: NF rats. Matos cell in this photograph are classified as the first type (type I) (M1) and second type (type II) (M2) in this paper. That is, two Mato cells are overlapped. The cell boundary is indicated by arrows. D: NF rats. This figure shows vascular cells (s) and a apart of extension of Mato cell (M). In the Mato cell of second type (type II), expanded endoplasmic reticula and vesicles are seen. M; Mato cell, E; endothelial cell, SM; smooth muscle cell, D; dense body, J; tight junction. E: NF rats. From the luminal surface of this figure, endothelium, smooth muscle cell and dark Mato cells (type III) are illustrated. Microglia (Mg) with large dense body abuts on Mato cell. E; endothelial cells, D; dense body, SM; smooth muscle cell, M; Mato cell, Mg; Microglia. F: CF rats. This figure shows endothelial cells, smooth muscle cells, and pale Mato cell. Mato cell is pale in cytoplasm and contain inclusions of various size. Some of them are vacuolized. The nucleus, endoplasmic reticula and mitochondria take normal profiles. E; endothelial cells, SM; smooth muscle cell, M; Mato cell, C; collagen fiber. G: CF rats. This figure shows vascular cells and some parts of extension of Mato cell. Endothelial cell protrudes into vascular lumen and contain mitochondria with normal profiles. In the outside of smooth muscle cells, extensions of Mato cells are displayed. It contains lysosomal inclusions with intense matrices. Around it, collagen fibers are seen (arrows). E; endothelial cells, SM; smooth muscle cell, M; Mato cell, C; collagen fiber, P; pinocytotic vesicle, J; tight junction. H: CF rat. Mato cell is pale and somewhat swollen, and contains several inclusions with or without vacuoles. Endoplasmic reticula gather in some part of cytoplasm. Pinocytosis is seen along the cytoplasmic membrane of endothelium and Mato cell (arrows). Mitochondria in the smooth muscle cells are stained intensely. Collagen fibers are scattered surrounding the Mato cells. E; endothelial cells, SM; smooth muscle cell, M; Mato cell, ER; Endoplasmic reticula, P; pinocytotic vesicle, C; collagen fiber.

cytoplasm. The tight junctions between endothelial cells were poorly developed (Fig. 4D). The smooth muscle cells had elongated nuclei, and myofilaments in them appeared fine and feeble (Fig. 4D). The course of myofilaments was not discernible. Occasionally, mitochondria were swollen, and some dense bodies were scattered in the cytoplasm (Fig. 4D). Caveolae along the cytoplasmic membranes were not evident. However, smooth muscle cells were not always hypertrophied or were only somewhat hypertrophied.

c) Mato cells in CF rats. The general features of Mato cells were similar in all specimens of CF rats, unlike those in NF rats. Mato cells were slender or somewhat swollen, and extended cytoplasmic extensions surrounding vascular walls (Fig. 4B, F). The intensity of the cytosol was relatively low, and it was rather pale (Fig. 4B, F, H). Collagen fibers were clearly observed around the Mato cells. Their nuclei were oval and featured perinuclear chromatin. The Mato cells contained various numbers of defined round lysosomal inclusions (Fig. 4F–H), and most mitochondria had normal profiles. Sometimes, endoplasmic reticula gathered in some parts of the cytoplasm (Fig. 4H). Pinocytotic vesicles were frequently observed along the cytoplasmic membranes (Fig. 4H).

The ultrastructural findings noted above suggest that in CF rats, Mato cells were functional and normal in profile and contents. They were similar to the Mato cells in normotensive Wistar rats reported previously [1–3].

d) Vascular cells in CF rats. As depicted in Figs. 4G and H, endothelial cells were thin and somewhat intense in general. Their nuclei were occasionally elongated, or irregular in

shape. Pinocytotic vesicles frequently appeared along the luminal surface (Fig. 4G, H). Between the endothelial cells, definite tight junctions had developed, as shown in Fig. 4G. The smooth muscle cells were slender, and their nuclei were elongated (Fig. 4B). Myofilaments were well-defined and ran parallel to the long axis of cells (Fig. 4G, H). Occasionally, mitochondria were intensely stained, with irregular profiles.

Discussion

As reported previously, the unique perivascular Mato cells surrounding cerebral arterioles occasionally exhibit degeneration in aged Wistar rats (26 months after birth), though in middle-aged Wistar rats (within 12 months) no signs of regression could be detected in them [1]. It has been speculated that the degeneration of Mato cells in old rats is due to overloading of waste products in the cerebral microvessels and cerebral tissues, since the Mato cells are rich in ApoE and hydrolytic enzymes, and efficiently take up excessive lipidic and proteinaceous substances at a young age [2–4, 11–13].

Of interest as well, perivascular Mato cells tended to degenerate in Wistar rats fed vitamin E-deficient chow for 8 to 10 months [11], although the vascular cells appeared healthy. In vitamin E deficient rats, membranous system—cytoplasmic membrane, mitochondria—in Mato cells was broken and damaged. On the other hand administration of vitamin E prevented the regressive changes of Mato cells [12]. These findings suggested that increase in oxidative

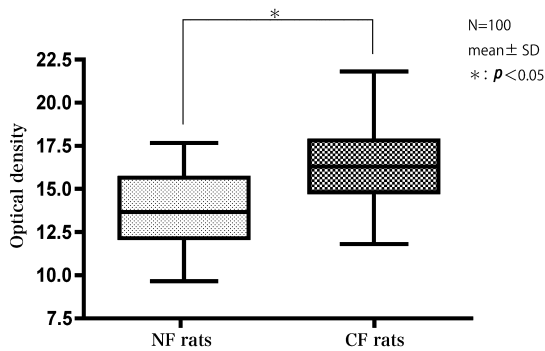


Fig. 5. This graph shows optical density of Mato cells in NF and CF rats after incorporation of HRP. OD value is calculated from 100 Mato cells.

stress had induced the degeneration of Mato cells.

To extend findings on Mato cells in hypertension, we employed SHR-SP(Izm) rats in this study. As illustrated in Fig. 1, the profiles of the Mato cells and intracellular lysosomal inclusions were clearer in CF rats than in NF rats. In previous studies [4, 5], inclusions in Mato cells were found to be rich in acid phosphatase and esterase, and stainable with PAS stain. The activity of acid phosphatase in the inclusions increased in parallel with stainability with the PAS method in young rodents [16, 17].

Secondly, to evaluate the principal function of Mato cells, the capacities for uptake of HRP of Mato cells in NF and CF rats were examined. As shown in Fig. 2 and Fig. 5, reaction products of HRP in CF rats were more evident than in NF rats. The deposition of HRP in CF rats attained to a similar level to that of wild type rodent [2, 4]. On the other hand in NF rats, capacity for uptake was thus markedly decreased. Photometric analysis using 100 Mato cells in each group confirmed the increase in uptake of HRP in CF rats (Fig. 5).

Histochemically, the activity of acid phosphatase in Mato cells of NF rats was compared that in CF rats, and findings suggested that it was remarkably higher in CF rats than in NF rats (Fig. 3). As described elsewhere [2, 4, 17], the activity of ACPase in Mato cells decreases with various conditions such as cerebral damage, aging, hypertension and scavenger receptor knockout, compared with that of wild rodents. So that, ACPase activity of Mato cells assumed to be correlated with uptake capacity and one of functional parameter of Mato cell.

These findings confirmed that the uptake capacity and activity of acid phosphatase of Mato cells in NF rats had deteriorated at 14 weeks of age and, in parallel with this, the intensity of ED2 was reduced, while ingestion of cocoa in CF rats prevented the deterioration of function of Mato cells seen in NF rats. However, the quantity of ingested cocoa employed in this study did not affect systolic blood pressure.

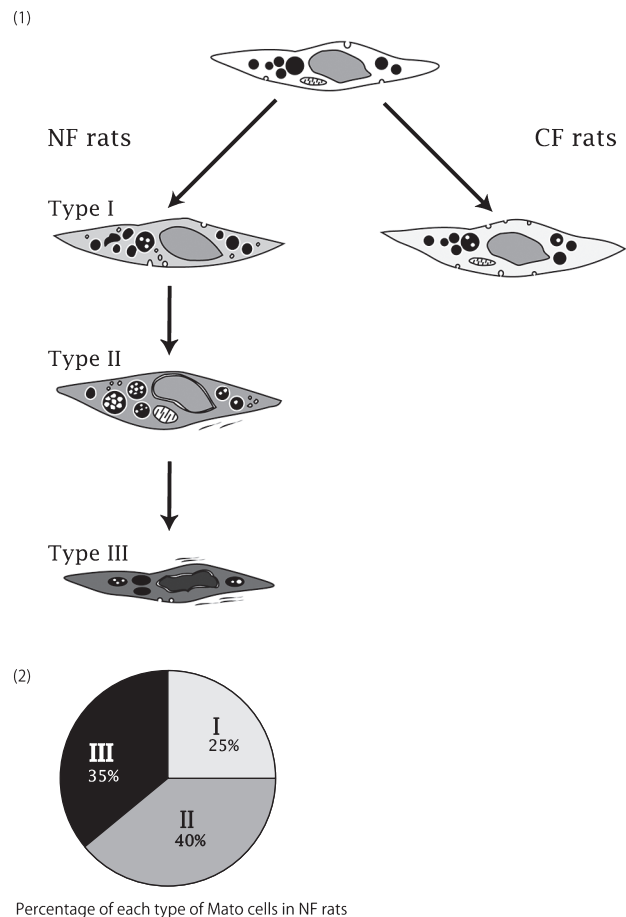


Fig. 6. (1) Schematic presentation of Mato cells (type I to type III) in NF rats and in CF rats. (2) Circular graph shows percentage of Mato cells in each type of NF rats.

Thirdly, ultrastructural study on cerebral arterioles and Mato cells was performed to clarify the pathological differences between NF and CF rats. At the electron-microscopic level, the Mato cells in NF rats varied in content and intensity. Tentatively, based on observations of the features of 100 Mato cells, Mato cells in NF rats were classified into three types, referring to nuclear shape, number of pinocytotic vesicles, swelling of mitochondria, expansion of endoplasmic reticula, number and size of lysosomal inclusions, and electron-density of the cytosol. Percentages by type were 25%, 40%, and 35% respectively, with type 2 a transitional type between types 1 and 3 (Fig. 6). All types of Mato cells in NF rats displayed regressive changes of different degrees, as described in the ultrastructural observations, though the most notable type of Mato cell belonged to the third type (Fig. 4A, E). The cells of this type were markedly intense, and appeared to have completely degenerated. We speculate that injurious products of hypertension induced impairment and degeneration of Mato cells.

However, Mato cells of CF rats fed chow containing cocoa appeared pale and somewhat swollen in the wide

perivascular space, and possessed defined lysosomal inclusions of various sizes and intensity (Fig. 4F, H). Their mitochondria had retained normal profiles. However, the Mato cells in CF rats were rich in pinocytotic vesicles, and endoplasmic reticula had gathered in some parts of the cytoplasm (Fig. 4H). The Mato cells in CF rats thus appeared to be viable, judging from the properties of cytoplasmic organelles, ACP activity and the intensity of the cytosole. Their profiles and lysosomal inclusions appeared similar to those of active Mato cells of young normotensive Wistar rats and humans reported elsewhere [1, 4, 5].

In parallel to the regressive changes of Mato cells in NF rats, the vascular cells also exhibited some pathological features. As shown in Fig. 4A, D, E in NF rats, endothelial cells were thin and had somewhat intense cytoplasm, and tight junctions between endothelial cells were not discernible. Pinocytotic vesicles were small in number, and dense bodies appeared frequently in them. The smooth muscle cells included fine and weak myofilaments arrayed in random directions. On the other hand, in CF rats, most endothelial cells were less intense, and occasionally the tight junctions between endothelial cells were well developed. Pinocytotic vesicles appeared frequently along the luminal surface. In smooth muscle cells, myofilaments ran parallel to the long axis. In NF and CF rats, smooth muscle cells appeared somewhat hypertrophic compared with those of normotensive Wistar rats [1].

Our findings suggest the hypothesis that degeneration of Mato cells and decrease in viability of vascular cells were associated with generation of superoxides due to hypertension in SHR-SP (Izm) rats. Concerning the relationship between hypertension and generation of superoxides, Rajagopalan [14] reported that infusion of angiotensin II induced increase in oxidative activity via membrane NADH/NADPH oxidase activation. Zafari *et al.* [15] and Zalba *et al.* [13] showed that overactivity of these enzymes was associated with impairment of NO-dependent relaxation of vessels and medial hypertrophy in the aorta of adult SHR rats. However, hypertrophy of the media was not as clear in the cerebral arterioles as in the aorta in this study. Furthermore, Chen *et al.* [18] reported that antioxidant effects of vitamins C and E were associated with the activity of vascular NADPH oxidase and superoxide dismutase in SHR-SP rats, and that these vitamins reduced oxidative stress driven by NADPH oxidase and improved vascular function and structures. Their results supported in part our previous findings concerning relationship between vitamin E deficiency and Mato cell [12].

As well known, Cocoa contain various types of antioxidants such as polyphenols. Sies *et al.* [19] reported that ingestion of flavonoid (flavan-3-ols), reversed the endothelial dysfunction in human subjects with cardiovascular disease through enhancement of NO bioactivity. According

to Abd El Mohsen *et al.* [20], epicatechin of cocoa crosses the blood-brain barrier, and protected against the neural cell degeneration induced by oxidative stress. Referring to their reports, it was expected that cocoa had beneficial effects against oxidative stress.

Based on the functional and morphological findings of this study, we conclude that generation of reactive oxygen species in SHR-SP (Izm) rats might impair Mato cells and vascular cells of the cerebral arterioles, and that although Mato cells are separated from endothelial cells and smooth muscle cells, they are highly vulnerable to generation of superoxides. However, ingestion of cocoa protects against injury of Mato cells and vascular cells in NF rats.

The precise relationship between dysfunction of Mato cells and pathological findings in vascular cells, as well as the generation of superoxides by Mato cells themselves require further study.

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