Effects of Colchicine on Amoeboid Microglial Cells in the Postnatal Rat Brain

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Received April 23, 1997; revised September 30, 1997

Summary. The present study was conducted to examine the response of amoeboid microglial cells in the postnatal rat brain to colchicine administration. One-day-old postnatal rats were given intraperitoneal injections of colchicine and sacrificed at 7, 14 and 21 days of age. In rats killed at 7 days age, the number of OX-42, OX-18 and ED1 positive amoeboid microglial cells was considerably reduced when compared with the control rats. At 14 and 21 days, the number of cells immunoreactive with the above antibodies was comparable to that of the control rats. The intensity of the immunoreaction with the various antibodies was also comparable in colchicine injected and control rats. When rhodamine isothiocyanate (RhIC) was administered, amoeboid microglial cells emitted a bright fluorescence in control rats as well as in colchicine-injected rats, although in the latter, the number of RhIC labelled cells was considerably reduced. With the antibody bromodeoxyuridine a large number of stained cells were observed in the control rats. On the other hand, occasional labelled cells were recognized in colchicine-injected rats. Apoptotic amoeboid microglial cells were observed in 4-day-old colchicine-injected rats. At the electron microscopic level, amoeboid microglial cells in colchicine-injected rats killed at 7 days of age showed a large number of phagosomes in their cytoplasm compared with the corresponding control rats. At 14 and 21 days, in colchicine-injected and control rats, amoeboid microglial cells did not display any noticeable differences. It is concluded from the present study that colchicine suppresses the number of amoeboid microglial cells, and that this may be attributed to the antimitotic effect of the drug as well as apoptosis induced by it; the phagocytic activity, however, was not affected. The cells returned to their normal population and morphological features once the drug was discontinued, indicating the reversible nature of the drug effect.

Amoeboid microglial cells are present in various regions of the central nervous system in the postnatal rat brain (LING, 1981; SIEVERS et al., 1981; KAUR et al., 1985; 1989; XU and LING, 1994). These cells are considered to be active macrophages in the developing brain (LING, 1977; KAUR et al., 1986; XU et al., 1993). Recently we investigated the effect of some drugs, e.g., glucocorticoids and chloroquine on the amoeboid microglial cells in the postnatal rat brain (KAUR et al., 1994, 1996), since these drugs are known to induce changes either in the number or morphology of macrophages (RUSSO MARIE, 1992; OHKUMA and POOLE, 1981). In addition to these, colchicine, a microtubule depolymerizing agent (ALLEN et al., 1991; GRUNSPAN-SWIRSKY and PICK, 1994) and an antimitotic drug (XU et al., 1992), has also been reported to reduce the number of macrophages in the brain at the site of injury (GUILIAN et al., 1989) in addition to inducing apoptosis (TSUKIDATE et al., 1993). The aim of the present study was to examine the effect of colchicine on the number of amoeboid microglial cells and to find out whether the drug would alter the expression of CR3 (complement type 3) receptors and major histocompatibility class I (MHC I) antigens constitutively expressed by these cells (LING et al., 1990, 1991). Since colchicine has been described as an inhibitor of phagocytosis (PRONAI et al., 1991), another aim of the present study was to assess whether the phagocytic activity of the amoeboid microglial cells is reduced following the administration of colchicine by using rhodamine isothiocyanate (RhIC), as these cells are known to endocytose RhIC in circulation in normal postnatal rats (XU et al., 1993). The present study also aimed to find out whether colchicine induced apoptosis of the amoeboid microglial cells and to examine the lyso-
somal enzyme acid phosphatase, as the lysosomal function is reported to be impaired following its administration (STROLINSKAIA and KOROVOKIN, 1991).

**MATERIALS AND METHODS**

One-day old postnatal Wistar rats were used in the present study. The rats (n=24) were given daily intraperitoneal injections (i.p.) of colchicine (0.2 mg/kg body weight; Sigma Co.) dissolved in saline for 6 days. Control rats (n=24) were administered an equal volume and frequency of saline injections. The rats were sacrificed at 7, 14 and 21 days of age. Another group of rats received daily i.p. injections of colchicine (n=4) or saline (n=4) as above for 3 days and were sacrificed at 4 days of age to detect apoptosis as described later.

To ascertain the phagocytic activity of amoeboid microglial cells following colchicine treatment, two colchicine-injected rats and their corresponding control were given a single intraperitoneal injection of 50 μl of 1% rhodamine isothiocynate (RhIC) in normal saline on the 6th day. The rats were perfused at 7 days of age.

To assess the effect of colchicine on the mitotic activity of amoeboid microglial cells, two of the colchicine-injected rats and their corresponding controls were given a single intraperitoneal injection of 50 μl bromodeoxyuridine (Sigma Co.) dissolved in saline (10 mg/ml) on the 6th day. The rats were sacrificed at 7 days of age.

**Immunohistochemistry**

Twelve rats injected with colchicine were sacrificed at 7 (n=4), 14 (n=4) and 21 days (n=4) of age; the same number of control rats were used at each time.
interval. They were perfused under deep ether anaesthesia with an aldehyde fixative composed of a mixture of periodate-lysine-paraformaldehyde with a concentration of 2% paraformaldehyde according to the method by MCLEAN and NAKANE (1974). After the perfusion, which lasted about 15 min, the brains were removed and fixed in a similar fixative for another 2 h and kept in 0.1 M phosphate buffer containing 10% sucrose overnight at 4°C. Frozen coronal sections of the brains were cut at the level of optic chiasma at a 40 μm thickness and rinsed in phosphate buffered saline (PBS). The sections were then incubated with one of the following monoclonal antibodies: OX-42 (Sera lab MAS 370b), OX-18 (Sera lab MAS 101b) diluted 1:100 with PBS, and ED1 (Serotec, MCA 341) diluted 1:400 with PBS. These antibodies recognize CR3 receptors, MHC class I antigens and cells of the monocyte/macrophage lineage, respectively. Incubation time with the above antibodies was between 18 and 24 h at room temperature. Subsequent antibody detection was carried out using Vectastain ABC-Kit (PK-4002, Vector Laboratories, Burlingame, CA) against mouse IgG with 3,3-diaminobenzidine (DAB, Cat No. 5637; Sigma Co., Mo) as a peroxidase substrate and intensified with nickel ammonium sulphate.

For quantitative study, three colchicine-injected rats and three control rats from each of the above age groups were used. OX-42 positive amoeboid microglial cells in the supraventricular corpus callosum as outlined by an ocular grid measuring 0.030 mm² were enumerated. A total area of 0.60 mm² was examined in each rat. The results of cell counts are summarized in Table 1.

Rats receiving colchicine and RhIC injections and their corresponding controls were perfused as above for immunohistochemistry, and coronal frozen sections of the brain of 30 μm thickness were cut and
mounted on gelatinized slides, air dried and cover slipped with non-fluorescent medium Entellan (Merck, Germany). The sections were examined and photographed in a Leitz Aristoplan photomicroscope equipped with a mercury lamp for fluorescence microscopy using a wide-band ultra-violet excitation filter (excitation range 515-560 nm). Adjacent serial sections were incubated with the OX-42 antibody and processed as described above.

Rats which had received injections of colchicine and bromodeoxyuridine and their corresponding control were perfused as above for immunohistochemistry. Sections from the brains were incubated with the antibody antibromodeoxyuridine (Amersham) at a concentration of 1:1 according to the method by SVENSSON (1993). Adjacent serial sections were incubated with OX-42 and processed as described above.

To detect apoptotic cells, terminal deoxynucleotidyl transferase mediated dUTP-fluorescein nick end labelling (TUNEL) using in situ cell death detection kit (Boehringer Mannheim, Germany) was carried out. The kit was used according to the manufacturer's instructions. Briefly, 4 colchicine-injected rats and 4 control rats were perfused at 4 days of age with 4% paraformaldehyde. The brains were removed and 40 μm thick frozen sections of the brains at the level of the optic chiasma were cut. The sections were incubated for 45 min in the TUNEL reaction mixture (TUNEL label with TUNEL enzyme; cat. No. 1767291 and 1767305, respectively). The sections were mounted and then viewed in a fluorescence microscope. After being photographed, the sections were incubated with the monoclonal OX-42 antibody and processed as above for immunohistochemistry.

Table 1. Number of OX-42 positive cells per 0.60 mm² area of the supraventricular corpus callosum in rats of various ages.

<table>
<thead>
<tr>
<th>Age</th>
<th>Colchicine-injected rats (mean±SD)</th>
<th>Control rats (mean±SD)</th>
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<tbody>
<tr>
<td>7 days</td>
<td>347.6±7.50</td>
<td>559.0±11.5</td>
</tr>
<tr>
<td>14 days</td>
<td>398.0±3.0</td>
<td>401.6±3.78</td>
</tr>
<tr>
<td>21 days</td>
<td>262.3±6.50</td>
<td>266.3±6.11</td>
</tr>
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Fig. 13. A large number of bromodeoxyuridine-labelled cells (dense dots) in the corpus callosum of a 7-day-old control rat. Scale bar = 50 μm

Fig. 14. Bromodeoxyuridine-labelled cells are markedly reduced in the corpus callosum of a 7-day-old colchicine-injected rat. Scale bar = 50 μm

Figs. 15 and 16. Corpus callosum of a 4-day-old colchicine-injected rat showing a number of cells emitting fluorescence with the TUNEL labelling method (Fig. 15) and showing immunoreactivity with the OX-42 antibody (Fig. 16). Some of the fluorescent cells (arrows in Fig. 15) are also OX-42 positive (arrows in Fig. 16). Scale bar = 30 μm

Fig. 17. Corpus callosum of a 7-day-old control rat showing a large number of acid phosphatase positive cells. The majority of the cells are round. Scale bar = 50 μm

Fig. 18. Corpus callosum of a 7-day-old colchicine-injected rat showing a few branched acid phosphatase positive cells. The acid phosphatase reaction in these cells is much weaker as compared with the cells in the control rats in Figure 17. Scale bar = 50 μm
To assess the effect of colchicine on the lysosomal enzyme acid phosphatase, two colchicine injected and two control rats were perfused with 10% formalin at 7 days of age. The brains were removed and 40 µm thick frozen sections at the level of the optic chiasma were cut. The sections were incubated and processed according to Gomori's method (PEARSE, 1968).

**Electron microscopy**

Six rats receiving colchicine injections and their corresponding control were sacrificed at 7 (n=2), 14 (n=2) and 21 (n=2) days of age. They were perfused under deep ether anaesthesia with Ringer's solution until the liver and lungs were clear of blood. This was followed by perfusion with a fixative composed of a mixed aldehyde solution of 2% paraformaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer. After perfusion the brains were removed and fixed in a similar fixative for another 2 h. Vibratome sections, 100 µm thick, of the brains were cut coronally at the level of the optic chiasma. The sections were post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer. They were then dehydrated and embedded in Araldite mixture. Ultrathin sections stained with lead citrate only were viewed in a Philips 120 CM electron microscope.

**RESULTS**

In rats receiving colchicine injections and sacrificed at 7 days of age, there was a drastic reduction in the number of OX-42 immunoreactive amoeboid microglial cells in the corpus callosum when compared with the corresponding control animals (Figs. 1, 2). The majority of OX-42 positive cells in the control animals were round, with a few cells showing short, thick processes, whereas in colchicine-injected rats most of the cells became ramified (Figs. 3, 4). Despite the ramification, the intensity of the immunoreactivity of microglial cells did not appear to be reduced. At 14 and 21 days of age, the OX-42 positive cells in colchicine-injected and control rats displayed similar external morphology, i.e. all cells were ramified showing comparable immunoreactivity (Figs. 5, 6). The number of OX-42 positive cells did not show any significant reduction in colchicine-injected and control rats at 14 and 21 days of age.

The results with the OX-18 antibody paralleled those with OX-42. Most of the labelled cells in colchicine-injected rats killed at 7 days of age were elongated and branched whereas in the corresponding control rats, most of the cells were round (Figs. 7, 8). At 14 and 21 days of age, all OX-18 positive cells either in colchicine-injected or control rats were branched, showing weak immunoreactivity.

The ED1 antibody results were similar to those with OX-42 and OX-18 in 7-day-old rats (Figs. 9, 10). However, at 14 days of age, only a few elongated ED1 positive cells were observed in the corpus callosum in the colchicine-injected rats as well as in the control rats. ED1 positive cells were absent in 21-day-old colchicine-injected and control rats.

The quantitative study showed that the number of OX-42 positive cells in the corpus callosum in 7-day-old rats was drastically reduced in colchicine-injected rats when compared with the control rats (Table 1). In 14 and 21-day-old rats, the number of OX-42 cells in the colchicine-injected and control rats was comparable (Table 1).

In control rats given a single injection of RhIC and killed at 7 days of age, a large number of amoeboid microglial cells emitting bright fluorescence were observed in the corpus callosum (Fig. 11). In the corresponding colchicine-injected rats, the number of RhIC labelled cells was considerably reduced (Fig. 12). The immunoreactivity with OX-42 appeared similar in both groups of rats following RhIC injections, although the number of immunoreactive cells in colchicine-injected rats was reduced drastically.

With the antibody against bromodeoxyuridine, numerous labelled cells were observed in the corpus callosum of control rats, whereas in the colchicine-injected rats only occasional labelled cells were observed (Figs. 13, 14). In the adjacent sections stained with OX-42, immunoreactive cells were noticeably reduced in the colchicine-injected rats when compared with the control rats (data not shown).

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**Fig. 19.** An amoeboid microglial cell in the corpus callosum of a 7-day-old control rat. The nucleus (N) shows dense chromatin clumps. The cytoplasm shows a large number of lysosomes, mitochondria, a few vacuoles (V). Golgi complex (G) and cisternae of rough endoplasmic reticulum. Scale bar = 1 µm

**Fig. 20.** An amoeboid microglial cell in the corpus callosum of a 7-day-old colchicine-injected rat showing a nucleus (N) with dense chromatin clumps. The cytoplasm shows very few lysosomes as compared with those in the cells of the control rats. Many phagosomes (P) can be seen in the cytoplasm. Scale bar = 1.5 µm

**Fig. 21.** A microglial cell in a 21-day-old colchicine-injected rat showing its nucleus (N) with dense chromatin masses. A small amount of cytoplasm containing a Golgi complex, lysosomes, mitochondria and cisternae of rough endoplasmic reticulum can be seen. Scale bar = 1 µm
Figs. 19-21. Legends on the opposite page.
With the TUNEL method, no fluorescent cells were observed in the corpus callosum of the 4-day-old control rats although a large number of OX-42 cells were present in the same region. In colchicine-injected rats, a large number of cells showed fluorescence in the corpus callosum (Fig. 15). Some of these fluorescent cells showed immunoreaction with OX-42 (Fig. 16).

A large number of round amoeboid microglial cells in the corpus callosum showed intense acid phosphatase activity in 7-day-old control rats (Fig. 17). In colchicine-injected rats, a few branched cells showed weak acid phosphatase activity (Fig. 18).

Electron microscopy

Amoeboid microglial cells in 7-day-old control rats showed a round nucleus with dense chromatin clumps at the periphery. The abundant cytoplasm contained numerous lysosomes, cisternae of rough endoplasmic reticulum, lipid droplets, some vacuoles and a Golgi complex (Fig. 19). In colchicine-injected rats the cells showed fewer lysosomes (Fig. 20). A common feature following colchicine treatment was the occurrence of large phagosomes in their cytoplasm (Fig. 20). In 14 and 21-day-old control and colchicine-injected rats, the amoeboid microglial cells displayed similar features. The cells were oval or elongated with a small amount of cytoplasm showing a few lysosomes, cisternae of rough endoplasmic reticulum and a Golgi complex (Fig. 21). Phagosomes were rarely observed in these cells.

**DISCUSSION**

The present study has demonstrated a considerable reduction in the number of amoeboid microglial cells in the corpus callosum in 7-day-old rats following the administration of colchicine. As shown by immunohistochemistry, use of the monoclonal antibodies OX-42, OX-18 and ED1 not only reduced the number of amoeboid microglial cells following colchicine injections but most of the labelled cells also appeared elongated and remified. Since colchicine is an antimitotic drug (Xu et al., 1992), it is possible that, following its administration, the mitotic activity of the amoeboid microglial cells is inhibited in the postnatal brain. Amoeboid microglial cells show active proliferation (Ling, 1981) which contributes to their steady increase in number from birth to the second week postnatally (Ling and Tan, 1974; Ling, 1981). During the same period, some of the amoeboid microglial cells undergo spontaneous degeneration while the surviving cells transform into ramified microglia (Ling, 1981; Kaur et al., 1985; Kaur and Ling, 1991). Following colchicine administration in the present study, only a few cells showed immunoreactivity with the antibody bromodeoxyuridine, compared with those in the control rats. Since the antibody is known to mark the dividing cells (Svensson, 1993), it is suggested that the mitotic activity of amoeboid microglial cells was impeded by the drug.

Colchicine is a microtubule disrupting drug (Tsukidate et al., 1993; Bonfoco et al., 1995) which induces apoptosis in different cell types, e.g. hepatocytes (Tsukidate et al., 1993) and cerebellar granule cells (Bonfoco et al., 1995). Colchicine administration in the present study appears to have induced similar changes, as apoptotic amoeboid microglial cells labelled with the TUNEL method and showing OX-42 immunoreactivity were observed in the present study. It could have also accelerated their degeneration which normally takes place in the postnatal brain (Kaur et al., 1985), leading to the reduction in number of amoeboid microglia in the corpus callosum. A large number of amoeboid microglial cells containing large phagosomes were observed in 7-day-old rats. The phagosomes might have been derived from the apoptotic or degenerated amoeboid microglial cells internalized by the surviving amoeboid microglial cells. Another reason for the reduced number of amoeboid microglial cells could be that colchicine treatment might have affected the entry of monocytes into the corpus callosum. Monocytes have been considered to be the precursors of amoeboid microglia (Ling, 1981). Colchicine is known to induce bone marrow depression leading to pancytopenia (Levy et al., 1991). It is possible that colchicine administration could also have suppressed the production of monocytes in bone marrow leading to an eventual reduction in the number of amoeboid microglial cells. In this connection, a reduced number of ED1 positive amoeboid microglial cells was observed in 7-day-old colchicine injected rats as compared with the control rats. ED1 recognizes cells of monocyte/macrophage lineage (Dijkstra et al., 1985).

Although colchicine has been described as a potent inhibitor of phagocytosis (Pronai et al., 1991), the phagocytic activity of the amoeboid microglial cells in the present study did not appear to be affected by the drug. This is evident from the uptake of RhIC by the amoeboid microglial cells as well as by the presence of a large number of phagosomes in them. The phagocytic activity of the amoeboid microglial cells is also evidenced by the expression of CR3 receptors which was comparable in both colchicine treated and control rats. CR3 receptors on the amoeboid microglial cells have been described as being involved...
endocytic activity (LING et al., 1990). One interesting feature in colchicine administered rats was the reduction of lysosomes in amoeboid microglial cells, consistent with the finding by STROLINSKAIA and KOROVOVIN (1991). A reduced number of lysosomes could have interfered with the digestion of the phagocytosed material leading to the presence of large phagosomes in the amoeboid microglial cells. This could also be due to a lesser amount of hydrolytic enzymes in the lysosomes. Acid phosphatase activity in the amoeboid microglial cells in the present study was weaker in the colchicine-injected rats as compared with the cells in control animals. Normal function of the lysosomes is reported to be impaired following colchicine administration which disrupts the microtubules. It has been suggested that microtubules play an important role in lysosome functioning (STROLINSKAIA and KOROVOVIN, 1991).

Previous studies have reported that the round amoeboid microglial cells transform into branched microglial cells by the second postnatal week (KAUR et al., 1985; KAUR and LING, 1991). In this connection, it is remarkable that colchicine administration appeared to bring about an early differentiation of amoeboid microglial cells since most of the cells in 7-day-old colchicine injected rats had transformed into the ramified form, while in the control rats, the cells remained round and amoeboidic—characteristics considered to be the nascent form of the cell type. Macrophages in culture have been shown to assume irregular profiles following depolymerization of microtubules using colchicine (ROSANIA and SWANSON, 1996).

Colchicine is known to decrease the expression of some cell surface antigens on the polymorphonuclear cells, e.g. the CR3 receptors (MISHIMA et al., 1992). The CR3 receptors and other surface antigens (MHC class I) did not appear to be reduced on the amoeboid microglial cells since most of the cells in 7-day-old colchicine injected rats had transformed into the ramified form, while in the control rats, the cells remained round and amoeboidic—characteristics considered to be the nascent form of the cell type. Macrophages in culture have been shown to assume irregular profiles following depolymerization of microtubules using colchicine (ROSANIA and SWANSON, 1996).

The immunoreactivity of amoeboid microglial cells with the antibodies OX-42, OX-18 and ED1 at 14 and 21 days of age was similar in colchicine-injected and control rats. In both groups of rats, the immunoreactivity with OX-18 was weak at 21 days of age, whereas with ED1, no immunoreactive cells were found. This is consistent with the earlier finding that MHC antigens are downregulated with age (LING et al., 1991) and that ED1 does not label the resting forms of microglia (MILLIGAN et al., 1991). Since amoeboid microglia transform into resting microglia by 2 weeks of age (LING, 1981; KAUR et al., 1985), ED1 immunoreactive cells were not found in 21-day-old rats in the present study.

The number of OX-42 and OX-18 immunoreactive cells was comparable in 14 and 21-day-old immunoreactive injected and control rats. It is possible that, with the discontinuation of colchicine administration, the mitotic activity of amoeboid microglial cells had returned to normal levels so that the number of amoeboid microglial cells was restored. It is also possible that with the withdrawal of the drug, more monocytes could have gained entry into the corpus callosum giving rise to new amoeboid microglial cells. Previous studies (LING, 1979) have shown that carbon labelled monocytes could enter the corpus callosum in the postnatal period and transform into amoeboid microglial cells. The effects of colchicine have been described as reversible (ARAKI et al., 1993).

Myopathies caused by colchicine have been described to regress with discontinuation of the drug (LE-QUINTEC and LE-QUINTEC, 1991).

Acknowledgements. The technical assistance of Mrs. ES YONG, Miss YG CHAN, Mr. Tajuddin bin M ALI and secretarial help of Mrs. Carolyne WONG is gratefully acknowledged. The author expresses deep gratitude to Professor EA LING for his valuable comments in the preparation of this manuscript.

REFERENCES


