¹H-MRI and *in vivo* ³¹P-MRS study of diseased state of smoky-brown cockroach infected with cockroach densovirus

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(Received: May 8, 1989)

Key words: ¹H-MRI, ³¹P-MRS, magnetic resonance spectroscopy, *Periplaneta fuliginosa*, cockroach densovirus.

Abstract: The nuclear magnetic resonance (NMR) technology was applied to the smoky-brown cockroach, *Periplaneta fuliginosa* (Serville), which had been infected with cockroach densovirus of the family Parvoviridae to correlate the diseased state with metabolic changes of the cockroach. ¹H-magnetic resonance imaging (MRI) revealed that, when abnormality is not yet recognized, increased spin lattice relaxation time (T_1) was detected in and around the midgut or hindgut region of the infected insects. ³¹P-magnetic resonance spectroscopy (MRS) demonstrated a decrease in the amount of arginine phosphate and, conversely, an increase in the amount of sugar phosphate, compared with uninfected insects.

INTRODUCTION

A viral agent, densovirus of the family Parvoviridae which causes a high incidence of hindgut ulcer, has been isolated from cockroach colonies (Suto *et al.*, 1978, 1979). When the cockroaches were injected with the virus, they lost movement coordination,

resulting in paralysis of the hind legs, swelling of the abdomen and enlargement of the fat body.

Pathophysiological as well as virological features of the virus-infected insects were thoroughly investigated by Suto *et al.* (1978, 1979); however, metabolic changes occurring in the infection process have not been known as yet.

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Recent advances in high-resolution nuclear magnetic resonance enabled us to study non-invasively the diseased state of animals. By using *in vivo* ^{31}P -MRS we have demonstrated marked differences in phosphorus metabolism between tumorous and normal brain of mice (Kato *et al.*, 1987).

In the present report, we describe the results of the pathophysiological study of virus-infected cockroaches, demonstrating significant differences in relaxation times of the water protons and also those in phosphorus metabolism between normal and virus-infected insects by means of ^1H -MRI and *in vivo* ^{31}P -MRS.

MATERIALS AND METHODS

Virus and cockroach. Cockroach densovirus was kindly provided by Dr. C. Suto. smoky-brown cockroach, *P. fuliginosa* (Serville), was a generous gift from Agricultural Chemical Research Laboratories, Sankyo Co., Ltd. They were fed water and dry food for mouse and rat (NM, Oriental Yeast Co., Ltd., Japan), and were maintained in a glass pot (25 cm ϕ $\times$ 20 cm) at $25\pm 1^\circ\text{C}$.

Virus inoculation. The crude virus solution was prepared according to Suto's method as described previously (Suto *et al.*, 1979). Briefly, the infected cockroaches were homogenized in phosphate buffered saline containing penicillin (500 U/ml), and streptomycin (100 $\mu\text{g}/\text{ml}$), and the homogenate was centrifuged twice at 10,000 *g* at 4°C for 20 min. Ten μl of crude virus solution (10% homogenate) was inoculated into the hemocoel (between the third and fifth abdominal segment) of healthy adults which had been anesthetized with ethyl ether. On the 3rd to 5th days after infection, the insects were subjected to *in vivo* ^1H -MRI and ^{31}P -MRS measurements.

In vivo ^1H -MRI and ^{31}P -MRS. Adults of *P. fuliginosa* were anesthetized with ethyl ether. Each insect was placed and fixed with plastic tape on a paper strip (2 $\times$ 20 cm), abdomen side up. For *in vivo* ^{31}P -MRS analysis, three insects were placed in a 25-mm ϕ tube with a Faraday shield as described previously (Kato *et al.*, 1987). The spectra were obtained at 109.14 MHz with a JNM-GX270 MR spectrometer from 600 transients

of 80 μsec (45°) delivered at 1.00 sec intervals. For ^1H -MRI (proton T_1) and -MRI analyses, a single insect was placed in a 25-mm ϕ tube and in an MRI probe, respectively. Coronal MR images were obtained by JEOL, NM-GIM270 in the following spectral parameters (echo time: 36 msec; repetition time: 2.0 sec; 256×256 points; slice thickness: 2 mm). For T_1 measurement, the data were obtained at 270 MHz in a Inversion Recovery mode (180° -t- 90° , 10 points).

RESULTS AND DISCUSSION

Water is the most abundant component of all living cells, existing as free or bound to protein, and has an important role in many biochemical and biophysical processes which occur in the cells. The structural and dynamic behavior of water in tissues with altered physiological state has been successfully studied by NMR technology (Hazelwood *et al.*, 1972). The T_1 can be related to molecular motility in tissues. Damadian (1971) found that water protons in a tumorous region have higher T_1 than in the corresponding healthy tissues. The increased T_1 is usually accounted for by the higher motional freedom of water molecules; higher water content or higher proportion of free water.

Table 1 summarizes T_1 of the water protons of *P. fuliginosa* uninfected and infected with densovirus. Considerable elongation of T_1 of infected male cockroaches was noted as compared with that of normal ones ($p<0.05$). In females, although T_1 tends to elongate in the infected insects, there was no statistical difference in T_1 between normal and infected cockroaches (Table 1). This difference, how-

Table 1 Comparison of the T_1 of water protons between normal and virus-infected cockroaches.

	T_1 of water protons (sec)	
	Uninfected	Virus-infected
Male	$0.716\pm 0.150^*$	1.046 ± 0.080
Female	0.698 ± 0.176	0.969 ± 0.324

* Mean $\pm$ S.D. in triplicate.

Fig. 1 MRI of *P. fuliginosa* infected with cockroach densovirus.

The color change from black toward white indicates the elongation of T_1 at the corresponding region. A: uninfected female; B: uninfected male; C: infected (5 days) female; D: infected (5 days) male.

ever, might be more significant if we increased the number of samples analyzed.

^1H -MRI was used to find the location of the lesion and to correlate the diseased state with metabolic changes. Figure 1 shows MR images, taken from abdomens by coronal slice, of uninfected and infected cockroaches (5 days). It clearly demonstrated that after the densovirus infection, T_1 in both male and female insects greatly increased in the localized region of abdomen, possibly midgut or hindgut. Similar results were obtained by changing slice positions for 2 or 3 mm back and front on the abdominal region of insects.

The observations obtained by MRI agreed well with the pathological finding that inflammation and necrosis are prominent in the midgut or hindgut of the infected insects

(Suto *et al.*, 1978). In support of this finding, we observed the midgut or hindgut region of the infected cockroaches on day 5 to be inflammatory or necrotic by an electron-microscope. Numerous virus particles of roughly 20 nm were present, showing the crystalline array in the cytoplasm of those infected gut cells (Takahashi, data not shown).

It is important to correlate morphological information with metabolic one. In this respect, ^1H -MRI and ^{31}P -MRS seem particularly suitable for this purpose.

Recently we have developed the Faraday shield method (Ohsaka *et al.*, 1983; Kato *et al.*, 1987) to obtain MRS spectra from a localized region of live animals. With this technique, we measured the *in vivo* ^{31}P -MRS

Fig. 2 *In vivo* ^{31}P -MRS spectra of virus-infected cockroaches.

A: uninfected control; B: infected (3 days after infection); C: infected (5 days after infection).

spectrum from the diseased, abdominal region of *P. fuliginosa* infected with densovirus (Fig. 2). As expected, phosphoarginine and ATP were clearly observed in the *in vivo* spectra (Fig. 2) as well as in the perchloric acid extracts of the cockroaches infected with densovirus (Takahashi, data not shown), indicating that acid extraction has been made adequately. The high-field resonances were easily assigned on the basis of data in the literature (Chin and Ellington, 1985) and by comparison with the chemical shift of the resonances of authentic samples. A major peak in the region of the spectrum to low field has been assigned to PME (phosphomonoesters). In many instances, these resonances are derived from various glycolytic intermediates (Barany and Glonek, 1984).

As shown in Fig. 2, in densovirus-infected cockroaches on day 5 or even on day 3, the amount of arginine phosphate decreased considerably while PME increased accordingly.

The results may be interpreted as indicating increase in glycolytic enzyme activities due to densovirus infection. We have observed similar changes in the brain tumor of mice (Kato *et al.*, 1987).

Identification of a diseased state by ^{31}P -MRS originates from the observation of Burt *et al.* (1976) that intact dystrophic chicken muscle contains an extra resonance not present in normal chicken muscle. This may, therefore, lead to the diagnosis of a disease correlated with a specific enzyme defect. Diseased states other than virus infection such as metabolic changes following drug treatment that result in an imbalance in energy metabolism, should all be amenable to investigation.

So far we have demonstrated ^1H - and ^{13}C -MRS are powerful tools for the quantitative study of carbohydrate metabolism in parasitic worms and insects (Nishina *et al.*, 1988; Takahashi *et al.*, 1987, 1989). The present study is an extension of application of NMR technology to virus-infected insects.

We now report for the first time the establishment of a model system for nondestructive measurement of the virus-infected live insects. In this study we have identified the location of the diseased state of densovirus-infected cockroaches by ^1H -MRI, and then observed changes in the phosphometabolism in the diseased region by ^{31}P -MRS. The present findings will pave the way for the basic understanding of physiology and biochemistry of medical insects.

ACKNOWLEDGEMENTS

The authors are grateful to Drs. F. Kawamoto, H. Fujioka and C. Suto, Department of Medical Zoology, Nagoya University School of Medicine, for providing cockroach densovirus and valuable suggestion, and also to Dr. H. Tsuji, Agricultural Chemical Research Laboratories, Sankyo Co., Ltd., for supplying *P. fuliginosa*.

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摘 要

デンソウウイルスを感染させたクロゴキブリの核磁気共鳴法による体内代謝の研究

核磁気共鳴法を用いて、デンソウウイルスを感染させたクロゴキブリ成虫の生体内代謝変化を調べた。1) 核磁気画像法 (^1H -MRI) で感染個体の中・後腸付近の T_1 値 (水のプロトン (^1H) 縦緩和時間) の増大が認められた。2) ^{31}P 核磁気共鳴スペクトロスコピー (^{31}P -MRS) で生体中のアルギニンリン酸と ATP のシグナルが非感染個体で明瞭に認められたが、感染個体では低下し、嫌気的狀態への体内代謝の変化が感染3日後から認められた。以上の結果から、デンソウウイルスを感染させたクロゴキブリは外見上何ら変化を認めなくても、個体の代謝系の変化が把握できた。