FULL PAPER *Pathology*

Sodium Iodoacetate-Induced Experimental Osteoarthritis and Associated Pain Model in Rats

Kiyoshi KOBAYASHI[1)#], Rei IMAIZUMI[2)], Hiroshi SUMICHIKA[2)], Hideki TANAKA[1)], Maki GODA[1)], Atsushi FUKUNARI[1)] and Hirotsugu KOMATSU[2)]

[1)]Discovery Technology Laboratory and [2)]Research Laboratory III, Research & Development Division, Mitsubishi Pharma Co., 1000 Kamoshida-cho, Aoba-ku, Yokohama 227–0033, Japan

(Received 24 April 2003/Accepted 22 July 2003)

ABSTRACT. Degenerative lesions were induced in the knee joint of Wistar rats by intraarticular injection of chondrocyte metabolism inhibitor mono-iodoacetate (MIA) at doses of 0, 0.3 or 3 mg/joint. Histopathological examination and the measurement of hind paw weight ratio as an index of joint pain by incapacitance tester were performed. Histological findings that are similar to those observe d in human osteoarthritis (OA), such as disorganization of chondrocytes, erosion and fibrillation of cartilage surface, and subchondral bon e exposure etc., were observed in a MIA-dose-dependent manner. Saflanin-O fast green staining revealed that marked diffuse reduction of proteoglycan in cartilage tissue of rats treated with MIA. The clinical scores of the joint pain were closely correlated to the grade of histological findings. We conclude that the present experimental model in combination with a novel dual channel weight averager would be very useful for the study of human OA, and could be applied for estimation of therapeutic effect of new anti-OA drugs.

KEY WORDS: mono-iodoacetate, osteoarthritis, pain assessment, rat.

J. Vet. Med. Sci. 65(11): 1195–1199, 2003

Osteoarthritis (OA) is a degenerative joint disease characterized by fibrillation and erosion in cartilage tissue, chondrocyte proliferation and osteophyte formation at the joint margins, and sclerosis of subchondral bone [13]. Reportedly, imbalance occurs between synthetic and degenerative process within chondrocytes that leads to the net loss of cartilage tissue and subsequent pathologic condition [2]. At late stage of human OA, articular damages eventually lead to clinical findings such as joint impairment and pain.

Although human OA-like lesions may occur spontaneously in dogs and mice, they are not appropriate for the evaluation of new anti-OA therapeutic agents because of low incidence and variable onset [5, 16]. There are number of animal OA models that has variety of etiologies such as surgical induction [11, 12, 15], collagenase-induced [9], extracellular matrix loss [18, 19], or impact-induced trauma [10]. However, studies on a new therapeutic drug for human OA and associated pain have been hampered because of the lack of useful animal model that closely mimic the human OA. Some study groups have previously reported that chondrocyte metabolism inhibitor mono-iodoacetate (MIA) have been reported to induce the disruption of glycolysis and subsequent cell death, and the loss of chondrocytes results in histologic changes in the knee joint resembling to human OA [6, 17]. The objective of the present study is to clarify the histopathologic changes in MIA-induced knee joint lesion in the rats and its correlation to the dose of MIA and clinical pain evaluated by dual channel weight averager, with a development trial of non-invasive rat OA model for new drug development.

[#)]PRESENT ADDRESS: KOBAYASHI, K., Pharmacology Department, Tsukuba Research Laboratories, Glaxo Smithkline K. K. 43 Wadai, Tsukuba 300–4247, Japan.

MATERIALS AND METHODS

Animals: Seven weeks old female Wistar rats were purchased from Charles River Japan Inc. (Yokohama, Kanagawa) and kept in air-conditioned animal room at 22°C and given tap water and basal diet. All animal experiments were conducted being in line with the guideline for animal handling and welfare in our facilities.

Treatment: Fifty μl of MIA (Wako, Tokyo) solution in saline at concentrations of 0, 6, or 60 mg/ml were intraarticularly injected in right knee, so that dose of MIA in each group was 0, 0.3, and 3 mg/joint, respectively. Left knee of each animal served as saline control.

Histological examination: Fifteen days after MIA injection, rats were anesthetized by diethyl ether and killed. Knee joint were removed and fixed in 10% neutral buffered formalin, decalcified by 10% formic acid, and embedded in paraffin. Five μm sections were stained by hematoxylin and eosin (HE) or saflanin-O fast green. Histopathological change of each animal was quantitatively expressed simply by the summation of individual grade (slight:1, moderate:2, severe:3) for each finding.

Pain assessment: The force exerted by each limb was measured dual channel weight averager (Linton Instrumentation, Norfolk, England) and joint pain was assessed by the distribution changes of weight between right and left hind paw (right/left paw weight ratio). Statistical analysis of paw weight ratio was performed by unpaired *t*-test using Microsoft Excel 2000 (Microsoft Japan, Tokyo).

RESULTS

Histopathology: In MIA-treated animals, joint cartilage

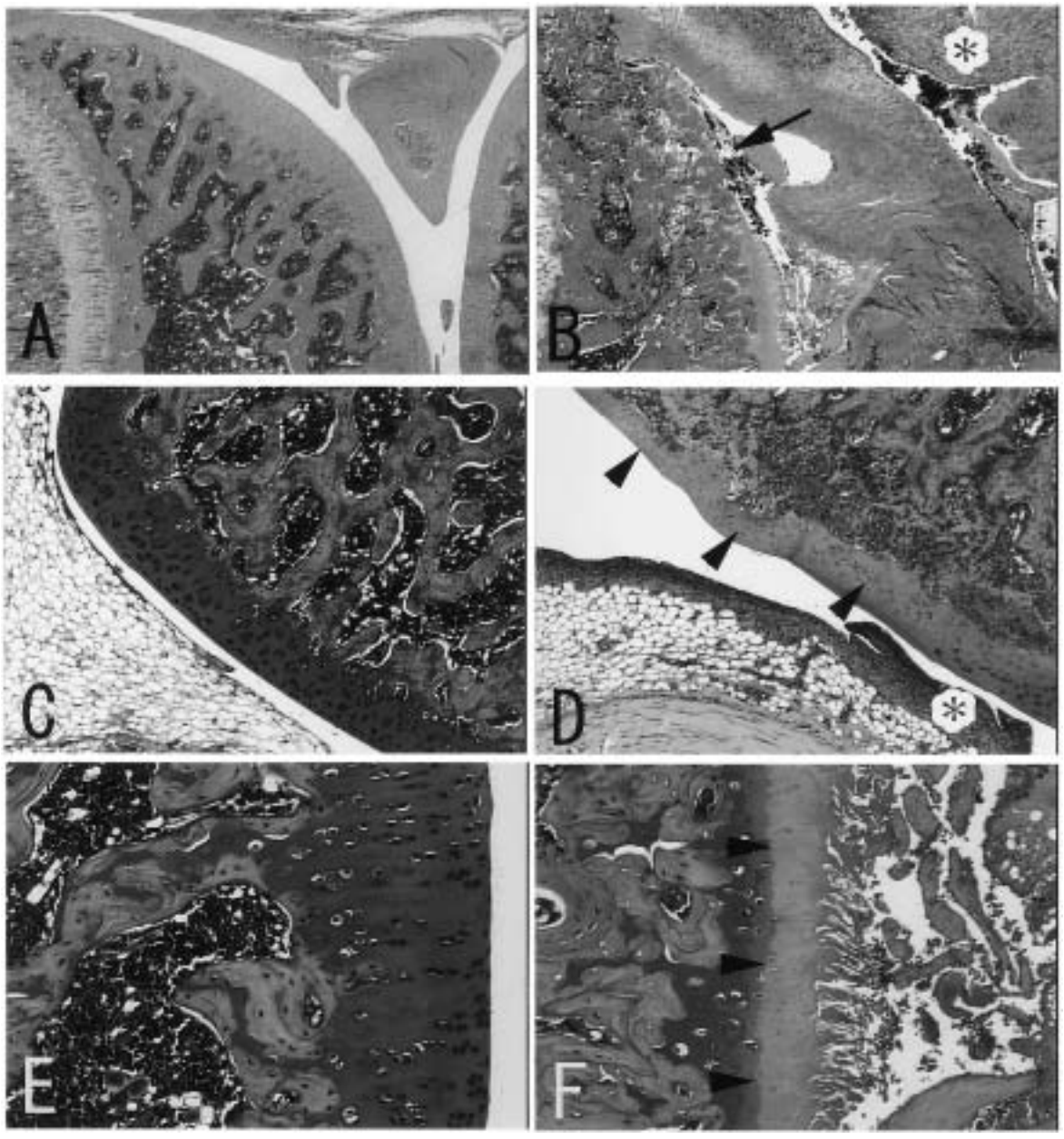

Fig. 1. Histopathological feature of osteoarthritic lesions in the knee joint (tibial bone) of rats treated with intraarticular injectio n of MIA. A, Saline control group. No abnormality is present. B, MIA-treated rat at a dose of 3 mg. Note the irregular surface of joi nt cartilage with focal erosion and exposure of subchondral bone (arrow). *: proliferated synovial cells with slight inflammatory c ell infiltration. C, Saline control group. No abnormality is present. Note the strong saflanophilia in normal cartilage tissue. D, M IA-treated rat at a dose of 3 mg. Marked, diffuse depletion of saflanin-O staining in joint cartilage (arrow heads). *: hyperplasti c synovial cells. E, saline control group. High magnification of joint cartilage tissue. F, MIA-treated rat at a dose of 3 mg. Marked fibrillation and the depletion of saflanin-O staining (arrow heads). A and B: HE stain, × 25. C and D: Saflanin-O fast red stain, × 50. E and F: Saflanin-O fast red stain, × 100.

of both femoral and tibial bone showed irregular surface that was occasionally accompanied by ulceration and/or fibrillation. Chondrocytes of joint caltilage were pale-staining, hypertrophied and disorganized that resulted in degeneration/necrosis. Regenerative hyperplasia of chondrocyte was not evident. These findings were clearly in a dose-dependent manner. Only in the high dose group (4 of 6 animals), the superficial layer of cartilage tissue was occasionally absent and exposure of subchondral bone was induced. Osteophyte was not present at the joint margins. Synovial

Table 1. Summary of microscopic findings

Treatment No. of Animals		Saline 5	MIA 0.3 mg/joint 5	MIA 3 mg/joint 6
Stractural changes in the joint				
Surface irregularities	+	0/5	4/5	0/6
	++	0/5	1/5	3/6
	+++	0/5	0/5	3/6
Average pathology score		0	1.2	2.5
Ulceration	+	0/5	1/5	0/6
	++	0/5	0/5	4/6
	+++	0/5	0/5	0/6
Average pathology score		0	0.2	1.3
Fibrillation of cartilage surface	+	0/5	1/5	4/6
	++	0/5	0/5	1/6
	+++	0/5	0/5	0/6
Average pathology score		0	0.2	1.0
Disorganization of chondrocytes	+	0/5	5/5	0/6
	++	0/5	0/5	6/6
	+++	0/5	0/5	0/6
Average pathology score		0	1.0	2.0
Exposure of subchondral bone	+	0/5	0/5	4/6
	++	0/5	0/5	0/6
	+++	0/5	0/5	0/6
Average pathology score		0	0	0.7
Cellular changes of chondrocyte				
Hypertrophy	+	0/5	5/5	1/6
	++	0/5	0/5	5/6
	+++	0/5	0/5	0/6
Average pathology score		0	1.0	1.8
Degeneration/Necrosis	+	0/5	4/5	0/6
	++	0/5	1/5	6/6
	+++	0/5	0/5	0/6
Average pathology score		0	1.2	2.0
Others				
Inflammatry cell infiltration	+	0/5	2/5	0/6
in synovial tissue	++	0/5	1/5	3/6
	+++	0/5	0/5	3/6
Average pathology score		0	0.8	2.5
Synovial cell proliferation	+	0/5	4/5	0/6
	++	0/5	0/5	2/6
	+++	0/5	0/5	4/6
Average pathology score		0	0.8	2.7
Saflanin-O staining				
Reduction of staining in cartilage	+	0/5	2/5	0/6
	++	0/5	3/5	0/6
	+++	0/5	0/5	6/6
Average pathology score		0	1.6	3.0
Total pathology score (average +/-S.E.)		0.0±0.0	8.0±1.4	19.3±1.26

+: Slight, ++: Moderate, +++: Severe.

cell of MIA-treated rats showed hyperplastic and moderately fibrotic changes accompanied by slight inflammatory cell infiltration. Saflanin-O staining revealed clear, diffuse depletion of proteoglycan in joint cartilage tissues of both femoral and tibial bone (Fig. 1). The depletion of saflanophilia was more pronounced in high dose group. Treatment-related change was not observed in saline-control animals and untreated left knee joint of MIA-given rats. Histopathological findings and pathology score are summarized in Table 1. Scoring of histological findings further confirmed the tendency of MIA-dose-dependent induction of various lesions (Table 1). Summation of all pathology finding

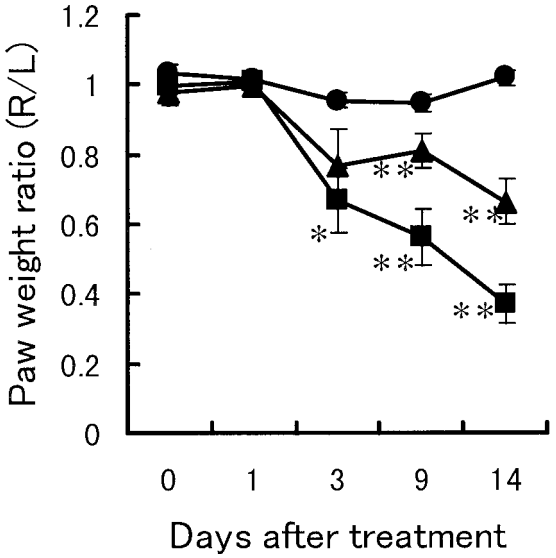

Fig. 2. Sequential observation of paw weight ratio. Right/left paw weight ratio is significantly decreased in MIA-treated in a dose-dependent manner. The change become severer with times post MIA injection. ●: Saline control, ▲: MIA 0.3 mg, ■: MIA 3 mg. * P<0.05, ** P<0.01 from control group.

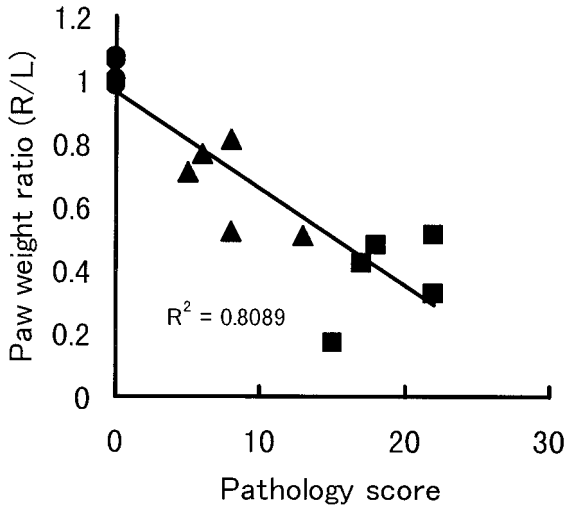

Fig. 3. Relationship between global pathology scores and average pain index for each animal at day 14. ●: Saline control, ▲: MIA 0.3 mg, ■: MIA 3 mg. Note the good correlation (R=0.8089).

scores in saline-control, low- and high-MIA dose group was 0.0 ± 0.0, 8.0 ± 1.4, and 19.3 ± 1.26, respectively (mean ± S.E.).

Pain assessment: Sequential analysis of joint pain revealed statistically significant reduction of right/left paw weight ratio in MIA-treated animals being in a dose-dependent fashion (Fig. 2). This change become severer with times post MIA injection. Global pathology change scores and average pain index of each animal at day 14 was plotted and showed good correlation (R=0.8089, Fig. 3).

DISCUSSION

MIA causes degenerative changes in articular cartilage by direct interference with chondrocyte metabolism [8] and has been used to induce degenerative joint disease in the rat [3], mouse [8], and guinea pig [1]. In the present study, MIA injection induced various histological changes closely resembled to human OA, e.g., ulceration and fibrillation of cartilage tissue accompanied by saflanophilic proteoglycan depletion and synovial cell reaction. These changes are mostly in line with the previous report by Guingamp [3] describing the knee joint changes of rats given MIA. Colombo *et al.* [1] have also reported human-OA like lesions in the rabbits induced by partial lateral meniscectomy. The lesions consisted of various degenerative changes including erosion and fibrillation of cartilage tissue with extensive loss of saflanin-O staining and are generally comparable to the present pathology findings. Exceptionally, osteophyte formation and the chondrocyte proliferation called “clone” or “cluster” which is known to be a characteristic pathology in human OA [1, 3, 13], were not seen in this study. These findings might be observed in somewhat severer condition or later stages in response to the exposure of subchondral bone tissue and the loss of chondrocytes. We tried to evaluate the pathology of the rats quantitatively by scoring the grade of each finding basically according to the method by Colombo *et al.* [1] that was applied to evaluate rabbit OA-model. As a result, a good correlation between pathology score and clinical signs was obtained. The fine reproducibility and quantitative potential, as well as very simple and rapid experimental procedure are considered to be the advantages of this experiment system to study the pathogenesis and anti-OA therapeutics.

One of the most important findings in the present study is both histological and clinical changes were observed clearly in MIA-dose-dependent fashion with relatively slight variation of the parameter among certain dose group. Since we would be able to modify the severity of the lesions by MIA dose, it will be useful for the experimental therapeutic study. In addition, it was noteworthy that saflanophilic proteoglycan, evaluated by simple saflanin-O staining, was markedly decreased in articular cartilage tissue. Since the loss of chondroid tissue is involved in the early stage of the pathogenesis of human OA [2], cartilage metabolism is a promising therapeutic target of human OA, and this experiment protocol would be useful and simply applied to the development trial of a new anti-OA, chondrocyte protecting drug.

We used dual channel weight averager to assess the joint pain expecting that it could provide an indication of weak perceived pain response. Normal rats distributes their body weight equally between the two hind paws, but when the right (MIA-treated, in this case) hind paw is painful, the rats re-distribute their body weight so that less weight is placed and right/left paw weight ratio would decrease. Likewise,

Hay *et al.* [4] and Tabo *et al.* [14] previously reported the measurement of weight-bearing by the hind limbs to analyze experimentally induced hyperalgesia and allodynia, using a equipment with two transducer that outputs the weight borne by right and left paw independently. Measurement of the hindlimb withdrawal threshold is a similar way to assess the joint pain [7], but it is not considered to be the evaluation of spontaneous pain. In this study, right/left paw weight ratio closely correlate to the degree of pathology in knee joint and significant changes in the pain index were seen only at and after 5 days after MIA injection. These results, as well as the fact that paw weight ratio measurement could be done both repeatedly and sequentially, suggest the usefulness of this novel equipment to measure clinically relevant pain.

Here we demonstrated that intraarticular injection of MIA induced human OA-like histopathologic changes and the loss of proteoglycan in cartilage tissue in the knee joint, clearly in a dose-dependent manner, and a dual channel weight averager provides a good pain index being correlated with the degree of pathologic changes. We conclude that this animal model in combination with dual channel weight averager offers a useful tool that could be applied for estimation of therapeutic effect of new anti-OA drugs. The evaluation of the efficacy of a new chondrocyte protect drug is in progress in our laboratory.

REFERENCES

1. Colombo, C., Butler, M., O'Byrne, E., Hickman, L., Swartzendruber, D., Selwyn, M. and Steinetz, B. 1983. A new model of osteoarthritis in rabbits. I. Development of knee joint pathology following lateral meniscectomy and section of the fibular collateral and sesamoid ligaments. *Arthritis Rheum.* **26**: 875–886.
2. Goldring, M. B. 2000. The role of the chondrocyte in osteoarthritis. *Arthritis Rheum.* **43**: 1916–1926
3. Guingamp, C., Gegout-Pottie, P., Philippe, L., Terlain, B., Netter, P. and Gillet, P. 1997. Mono-iodoacetate-induced experimental osteoarthritis: A dose-response study of loss of mobility, morphology, and biochemistry. *Arthritis Rheum.* **40**: 1670–1679.
4. Hay, C. H., Trevethick, M. A., Wheeldon, A., Bowers, J. S. and de Belleroche, J. S. 1997. The potential role of spinal cord cyclooxygenase-2 in the development of Freund's complete adjuvant-induced changes in hyperalgesia and allodynia. *Neuroscience* **78**: 843–850.
5. Hewicker-Trautwein, M., Carter, S. D., Bennett, D. and Kelly, D. F. 1999. Immunocytochemical demonstration of lymphocyte subsets and MHC class II antigen expression in synovial membranes from dogs with rheumatoid arthritis and degenerative joint disease. *Vet. Immunol. Immunopathol.* **67**: 341–357.
6. Kalbhen, D. A. 1987. Chemical model of osteoarthritis-a pharmacological evaluation. *J. Rheumatol.* **14**: 130–131.
7. Kauppila, T., Kontinen, V. K. and Pertovaara, A. 1998. Weight bearing of the limb as a confounding factor in assessment of mechanical allodynia in the rat. *Pain* **74**: 55–59.
8. van der Kraan, P. M., Vitters, E. L., van de Putte, L. B. and van den Berg, W. B. 1989. Development of osteoarthritic lesions in mice by "metabolic" and "mechanical" alterations in the knee joints. *Am. J. Pathol.* **135**: 1001–1014.
9. van der Kraan, P. M., Vitters, E. L., van Beuningen, H. M., van de Putte, L. B. and van den Berg, W. B. 1990. Degenerative knee joint lesions in mice after a single intra-articular collagenase injection. A new model of osteoarthritis. *J. Exp. Pathol.* **71**: 19–31.
10. Mazieres, B., Blanckaert, A. and Thiechart, M. 1987. Experimental post-contusive osteoarthritis of the knee: Quantitative microscopic study of the patella and the femoral condyles. *J. Rheumatol.* **14**: 119–121.
11. Moskowits, R. W., Davis, W., Sammarco, J., Martens, M., Baker, J., Mayor, M., Burstein, A. H. and Frankel, V. H. 1973. *Arthritis Rheum.* **16**: 397–405.
12. Pond, M. J. and Nuki, G. 1973. Experimentally induced osteoarthritis in the dog. *Ann. Rheum. Dis.* **32**: 387–388.
13. Poole, A. R., Rizkalla, G., Ionescu, M., Reiner, A., Brooks, E., Rorabeck, C., Bourne, R. and Bogoch, E. 1993. Osteoarthritis in the human knee: a dynamic process of cartilage matrix degradation, synthesis and reorganization. *Agents Actions* **39**: 3–13.
14. Tabo, E., Eisele, J. H. Jr and Carstens, E. 1998. Force of limb withdrawals elicited by graded noxious heat compared with other behavioral measures of carrageenan-induced hyperalgesia and allodynia. *J. Neurosci. Methods.* **81**: 139–149.
15. Vignon, E., Bejui, J., Mathieu, P., Hartmann, J. D., Ville, G., Evreux, J. C. and Descotes, J. 1987. Histological cartilage changes in a rabbit model of osteoarthritis. *J. Rheumatol.* **14**: 104–106.
16. Wilhelmi, G. and Faust, R. 1976. Suitability of the C57 black mouse as an experimental animal for the study of skeletal changes due to ageing, with special reference to osteo-arthrosis and its response to tribenoside. *Pharmacology* **14**: 289–296.
17. Williams, J. A. and Thonar, E. J. 1989. Early osteophyte formation after chemically induced articular cartilage injury. *Am. J. Sports Med.* **17**: 7–15.
18. Williams, J. M., Ongchi, D. R. and Thonar, E. J. 1993. Repair of articular cartilage injury following intra-articular chymopapain-induced matrix proteoglycan loss. *J. Orthop. Res.* **11**: 705–716.
19. Williams, J. M., Uebelhart, D., Thonar, E. J., Kocsis, K. and Modis, L. 1996. Alteration and recovery of the spatial orientation of the collagen network of articular cartilage in adolescent rabbits following intra-articular chymopapain injection. *Connect. Tissue Res.* **34**: 105–117.