

Pilot study of the effect of acemannan in cats infected with feline immunodeficiency virus

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ABSTRACT

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Acemannan, a complex carbohydrate shown to stimulate interleukin-1, tumor necrosis factor alpha and prostaglandin E₂ production by macrophages, has also demonstrated antiviral activity in vitro against human immunodeficiency virus, Newcastle disease virus and influenza virus. A pilot study was undertaken to determine acemannan's effect in 49 feline immunodeficiency virus (FIV) infected cats with clinical signs of disease (Stage 3, 4 or 5), 23 of which had severe lymphopenia. Cats received acemannan either by intravenous (Group 1) or subcutaneous (Group 2) injection once weekly for 12 weeks, or by daily oral (Group 3) administration for 12 weeks. Upon entry into the study, cats were randomly assigned to one of the three groups. Laboratory analyses were performed at the beginning of the study and at Weeks 6 and 12. Cats were allowed to continue with a predetermined maintenance regimen of acemannan after completing the 12-week study.

Thirteen cats died during the course of treatment. Upon necropsy, the most frequent histopathologic findings were neoplastic, kidney and pancreatic disease.

Friedman's two-way ANOVA test showed no significant differences in efficacy among groups administered acemannan by the different routes. Therefore, groups were combined and a signed-ranks test was used to determine changes over time. A significant increase was seen in lymphocyte counts ($P < 0.001$). Neutrophil counts decreased significantly ($P = 0.007$), as did incidence of sepsis ($P = 0.008$). When cats entering with lymphopenia were analyzed separately, a much greater increase in lymphocyte counts was noted (235%) compared with non-lymphopenic cats (42%).

A survival rate of 75% was found for all three groups. Thirty-six of 49 animals are alive 5–19 months post-entry.

These results suggest that acemannan therapy may be of significant benefit in FIV-infected cats exhibiting clinical signs of disease.

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ABBREVIATIONS

ARC, AIDS-related complex; CrFK, Crandall feline kidney (cells); FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; HIV, human immunodeficiency virus; IFA, immunofluorescent antibody; IL-2 interleukin-2.

INTRODUCTION

The feline immunodeficiency virus (FIV) was first isolated in 1986 (Pedersen et al., 1987) and has since been found worldwide. FIV shows a high degree of tropism for CD4+ T-cells and, as a result, induces a slowly progressive immunodeficiency syndrome. The natural progression of this disease has not yet been completely elucidated. It is clear, however, that bites from infected animals are the principal mode of transmission.

Following initial infection, cats exhibit signs of fever, neutropenia and generalized lymphadenopathy (Stage 1) (Pedersen and Barlough, 1991). In most cases these signs are mild and not likely to be brought to the attention of a veterinarian. Nevertheless, these cats may have a mortality rate of 5–20% per year (Pedersen, 1990). Most FIV-infected cats recover within a few weeks and become asymptomatic despite an underlying chronic infection (Stage 2). This asymptomatic period may last for several months to years, during which there is a slow, progressive decline in CD4+ cells (predominantly T-helper cells). Eventually, this progressive loss of helper cells leads to increased susceptibility to infection and the gradual onset of clinical disease.

The onset of clinically significant immunodeficiency is marked by development of a persistent, generalized lymphadenopathy (Stage 3) which leads inevitably to development of chronic secondary infections (Stage 4). In Stage 4 weight loss is common, and hematologic abnormalities occur in approximately one-third of infected cats. Infections occur with increasing frequency as immunosuppression develops. In Stage 5, animals suffer from chronic secondary and opportunistic infections of increasing severity until death eventually occurs (Pedersen and Barlough, 1991). Anemia and leukopenia are common findings in Stage 5. Cats in this stage usually survive for only 1–6 months regardless of treatment (Ishida and Tomoda, 1990).

Currently, no effective treatment other than supportive therapy exists for FIV-infected cats. A new complex carbohydrate, acemannan, is currently being investigated for treatment of retroviral diseases. Acemannan is a water-soluble, long-chain, β -(1,4)-linked mannan polymer with an average of one *O*-acetyl group per mannose residue. The compound is poly dispersed with molecular weights greater than 10 000 and an average molecular weight of approximately 1×10^6 .

Acemannan causes phenotypic activation of macrophages, enhancing both phagocytic activity and non-specific cytotoxicity (Measel and Denham, 1988)

as well as expression of Class II major histocompatibility complex antigens (Eldridge, 1990). Exposure of macrophages to acemannan in vitro results in the rapid synthesis of tumor necrosis factor alpha and interleukin-1 (IL-1) (Peng et al., 1991). Acemannan also enhances the response of human lymphocytes to alloantigen (Womble and Helderma, 1988). Other studies have shown that acemannan is a potent adjuvant when administered with antigen (Chinnah et al., 1991).

In addition to its effects on the immune system, acemannan has been shown to have a direct antiviral effect on lentivirus replication in vitro. Acemannan caused a concentration-dependent inhibition of human immunodeficiency virus (HIV) replication in CEM-SS cells infected with the RF_{II} strain of HIV-1. Viral load, cell-free virus, syncytium formation and cytopathic effects were all reduced following treatment of infected cells with acemannan. It is believed that the antiviral effects of acemannan are the result of altered oligosaccharide processing, leading to inhibition of viral replication (Kahlon et al., 1991).

In one study, six weekly intraperitoneal (IP) injections of acemannan in cats naturally infected with feline leukemia virus (FeLV) led to significant improvement in clinical status and survival. Twelve weeks after initiation of acemannan treatment 71% of treated cats were alive and in good health (Sheets et al., 1991).

Given the efficacy of acemannan in treating cats affected by FeLV, the study described herein was initiated to determine whether acemannan had any effect on clinically ill cats with FIV infection and to determine acemannan's optimal route of administration. Animals surviving at the end of the 12 week study were put on a maintenance regimen of acemannan to evaluate long-term clinical improvement and survival.

MATERIALS AND METHODS

Clinical population

Naturally infected cats exhibiting clinical signs of FIV infection were presented by their owners to three veterinary clinics in Texas. All were ELISA-positive and immunofluorescent antibody (IFA) positive for the infection. ELISA tests for FIV antibody were performed by a commercial veterinary reference laboratory and on location using the CITE^R (IDEXX, Westbrook, ME) test. IFA testing was performed by Carrington Laboratories, using Crandall feline kidney (CrFK) cells.

Informed consent

Owners of enrolled cats were required to have their pets treated with acemannan for the duration of the study and to acknowledge this agreement by

signing informed consent forms. They were also required to return their cats for all assigned visits and to permit necropsies should their animals die.

Experimental design

Upon entering the study, cats were randomly assigned to one of three treatment groups. Group 1 received 2 mg acemannan kg^{-1} intravenously (IV) once weekly. Group 2 received 2 mg acemannan kg^{-1} subcutaneously (SQ) once weekly. Group 3 received 100 mg acemannan orally (PO) daily. Cats were allowed to receive concomitant supportive therapy, i.e. antibiotics, vitamins, fluid therapy. Study duration was 12 weeks, but cats were permitted to continue on therapy if clinical improvement was noted. Cats treated post-study received acemannan by monthly injection (2 mg kg^{-1} ; Groups 1 and 2) or daily oral administration (100 mg; Group 3).

Injectable acemannan was provided in kits containing freeze-dried powder (10 mg) and sterile diluent (10 ml) for reconstitution. PO acemannan was provided in 100 mg capsules, the contents of which were mixed with food.

Clinical assessment

Treated cats underwent intensive clinical examination at screening, Week 6 and Week 12. Clinical indices included general condition and appetite, weight change, body temperature, and presence of sepsis or lymphadenopathy. Body weight at entry was used to determine subsequent body weight change.

Hematologic examination included determination of complete red blood cell counts, differential white blood cell counts, estimated platelet counts, hematocrit and hemoglobin.

Blood chemistry values measured included glucose, blood urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatine phosphokinase, total bilirubin, total protein, albumin, calcium, phosphorus, lactate dehydrogenase and cholesterol.

Necropsies were performed on all animals that died, and histopathologic examinations were performed on submitted tissues by a board-certified pathologist.

Adverse events

Adverse event data were obtained as the study progressed and by checklist summary at the conclusion of the study. Any adverse events associated with administration of acemannan were recorded by the clinician.

Statistical methodology

Using the median values for each test within each treatment group, Friedman's two-way analysis of variance by ranks was conducted to determine whether or not significant differences existed among the three groups. Friedman's test does not require the assumption of normality of the underlying distribution.

A rank transformation technique was used to detect treatment effects in a repeated measure design (Kepner and Robinson, 1988). Since this test investigates differences over time for any given index, only those animals with complete records were included.

The generalized Wilcoxon test and the Mann-Whitney test were used to analyze differences in survival among groups.

An alpha level of 0.01 was chosen to ensure significance of all test differences.

RESULTS

Study population

The study population comprised 49 clinically diseased, FIV-infected cats. Groups 1 and 2 (IV and SQ groups) consisted of 17 animals each; Group 3 (PO group) consisted of 15 animals.

Cats were both ELISA- and IFA-positive for FIV; one was Stage 3, 41 were Stage 4 and seven were Stage 5 (Table 1). Ages of animals ranged from 1.5 to 17 years with a median of 7 years. Forty males (82%) and nine females (18%) were treated. All female cats were spayed, and all but two males were neutered. Thirty-five of the 49 cats (71%) were Domestic Short Hair, 13 were Domestic Long Hair (27%), and one was purebred (2%). The most frequent clinical findings upon study entry were anorexia/weight loss, lymphopenia and oral mucosal pathology (Fig. 1).

TABLE 1

Staging at entry

Group	Stage 3	Stage 4	Stage 5	Total
1	0 (0%)	14 (28.6%)	3 (6.1%)	17
2	1 (2%)	15 (30.6%)	1 (2%)	17
3	0 (0%)	12 (24.5%)	3 (6.1%)	15
Totals	1 (2%)	41 (83.7%)	7 (14.2%)	49 (100%)

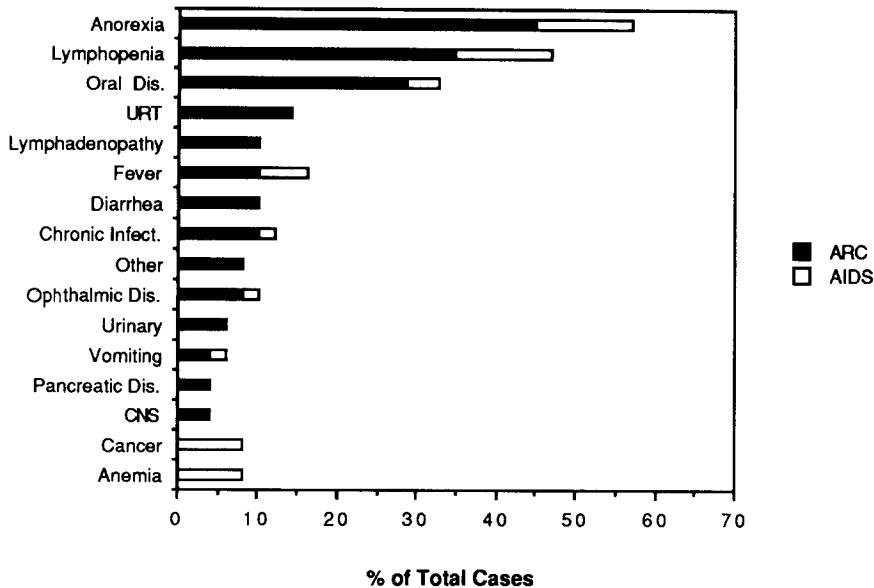


Fig. 1. Frequency histogram of primary clinical signs of FIV-infected cats upon study entry. The cases are divided into those animals presenting with AIDS-related complex (ARC; solid bars) and those with frank AIDS (open bars).

Clinical/statistical data

Statistical analyses of clinical scores, hematology and chemistry using Friedman's test showed no differences in efficacy among the three groups at Weeks 0, 6 or 12. Since a random distribution of disease and demographics existed among all three groups, they were combined to evaluate changes over time (Kepner and Robinson, 1988). A trend towards increase in body weight (as shown by percentage change) was noted ($P=0.02$), as were reductions in fever ($P=0.02$) and lymphadenopathy ($P=0.05$). While these changes were not statistically significant, a reduction in sepsis was significant ($P=0.008$).

Significant clinical changes occurred in several other hematologic parameters as shown in Table 2.

In the 23 cats that initially had lymphopenia, the median lymphocyte count increased from 809 at Week 0 to 1900 at Week 12 (235%; $P=0.001$). Neutrophils of lymphopenic cats dropped from 6320 to 4095 (35%; $P=0.006$).

Survival

Of the 49 cats entered in the study, nine died or were killed during the 12-week study. Four others died during post-study maintenance and surveillance.

The ability to follow animals past the 12-week study period facilitated long-

TABLE 2

Significant changes in hematology values

Parameter	Median Week 0	Median Week 6	Median Week 12	<i>P</i> value
Lymphocytes (cells mm ⁻³)	1280	2006	1815	0.001
Neutrophils (cells mm ⁻³)	6310	4900	4366	0.007
Hemoglobin (g dl ⁻¹)	12.1	11.8	11.0	0.008
Hematocrit (%)	35.6	31.1	31.3	0.002

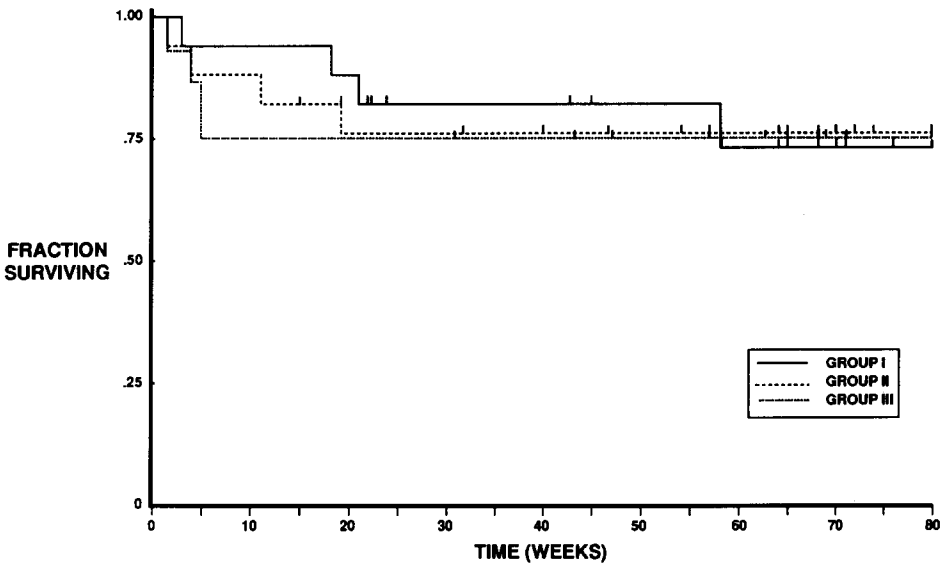


Fig. 2. Kaplan-Meier survival curves.

term survival analysis. Estimated probabilities of survival were calculated, and results are shown as Kaplan-Meier survival curves (Fig. 2). The majority of these animals are still living (5+ to 19+ months). There was not a statistically significant difference among the three groups in terms of survival as measured by the generalized Wilcoxon test. All treatment groups appear to converge at a surviving fraction of approximately 0.75. However, cats in Group 3 (PO) reached this value first (Week 5) followed by Group 2 (SQ) (Week 19). Animals in Group 1 (IV) had the best short-term survival, requiring 58 weeks to reach this level.

Median survival time of animals with Stage 4 (ARC) disease was 58 weeks ($n=41$), while median survival time of animals with Stage 5 (AIDS) disease was 11 weeks ($n=7$). This difference was found to be statistically significant by the Mann-Whitney test ($P=0.006$). No difference in survival times was

TABLE 3

Histopathology findings at necropsy

Case No. ¹	Tumors	Pancreatic disease	Musculoskeletal disease	Liver disease	Cardiovascular disease	Kidney disease	Immune system disease	Respiratory system disease	Endocrine system disease	Gastrointestinal disease	Necropsy diagnosis at stage
4		+		+		+					4
7		+		+		+		+			4
13	+ ²				+						5
28				+ ³			+				5
29	+ ⁴	+			+	+ ⁴	+	+		+ ⁵	5
33		+			+		+				5
41	+ ⁴						+				5
43				+			+				5
50	+ ⁶	+					+	+			5
52	+ ⁴					+		+ ⁶	+		5
59	+ ⁴	+	+ ⁴								5

¹Nos. 15 and 35 lost to follow-up.

²Adenocarcinoma.

³Hemosiderosis.

⁴Lymphosarcoma.

⁵Feline infectious peritonitis.

⁶Carcinoma.

found between animals that were lymphopenic at entry ($n=23$) and animals with normal lymphocyte counts ($n=26$).

Histopathology

Thirteen cats died during either the course of the study or post-study maintenance; two were lost to follow-up and 11 were necropsied (Table 3). Significant pathology of the pancreas, kidneys and respiratory system was noted. Pancreatic pathology included interstitial fibrosis, autolysis, exocrine atrophy, granulomatous perivascularitis, islet cell depletion and nodular hyperplasia. Chronic interstitial nephritis of the kidney was frequently found. In the respiratory system, chronic multifocal proliferative pneumonitis, pulmonary arteriopathy, alveolar emphysema, suppurative bronchopneumonia, and chronic fibrinous pleuritis were observed. Four animals had lymphoid depletion. Lymphosarcomas were found in four cats and carcinomas in two.

Adverse events

Adverse events were limited to four cats (24%) in the IV group. These animals experienced tachypnea and/or tachycardia, began to salivate profusely, and became limp or collapsed immediately after injection. Pale oral mucosae were observed in these animals.

The reactions in one of these cats decreased in severity with each treatment until, by the end of the study, reactions were considered very mild. Another cat was treated by IP injection for the remainder of the study without further incident. The remaining two cats were treated for the duration of the study by IV drip instead of IV push and experienced no further adverse reaction to treatment.

DISCUSSION

Clinical/statistical data

The ages of cats in the study were similar to those reported by Yamamoto et al. (1989) and Ishida et al. (1989) who found the average age of clinically infected cats to be 5–6 years. Sex differences in the study population existed with males having four times the frequency of infection of females. Disease signs were similar to those previously reported (Ishida et al., 1989; Yamamoto et al., 1989), with the exception of the high incidence of pancreatic involvement seen in animals in this study.

Nineteen of 49 cats (39%) had elevated serum glucose levels (> 150 mg dl^{-1}) upon entry; 11 of these (22%) had glucose levels of 200–500 mg dl^{-1} . The reason for these high levels is not known.

Several other changes were noted in laboratory values over the 12-week study period. A significant increase was observed in absolute lymphocyte count in general (42%), while in lymphopenic cats an even larger increase was seen (235%). This is important because lymphopenia is the most common hematologic manifestation in FIV-infected cats.

In previous studies, decrease in total lymphocyte count was related to a decrease in the T-cell population, mainly CD4+ T-helper cells (Novotney et al., 1990; Tompkins et al., 1991). While lymphocyte subset analysis was not performed here, it is interesting to speculate that the increase in lymphocyte counts might be partially attributable to an increase in CD4+ T-cells. It will be interesting to see if this trend of increasing lymphocyte counts continues as these animals remain on maintenance therapy.

Absolute neutrophil counts were found to decrease (approximately 30%) after acemannan therapy in cats both with and without lymphopenia. This normalization in neutrophil count presumably follows the noted decline in incidence of sepsis. Taken together with the rise in lymphocyte counts, it seems these animals were better able to control and eliminate infections after acemannan therapy. Macrophage activation induced by acemannan may have had a supporting role in helping to eliminate residual bacteria.

Acemannan has two recognized activities. First, it induces macrophage activation with subsequent synthesis of cytokines and enhances phagocytosis. Mannans have been reported to induce interferon synthesis *in vivo* (Tizard et al., 1989). It is therefore possible that acemannan induced production of interferon in these animals, which would inhibit viral replication. Similarly, the immune stimulation induced by acemannan, particularly IL-1 synthesis, may have resulted in some recovery of immune function. Macrophage activation could also enhance killing of virally infected cells via activation of cytotoxic T-cells.

Second, acemannan possesses antiviral activity by modifying glycosylation of both virally infected cells and glycoprotein coats of viruses, thus inhibiting virus replication and infectivity. This effect has been shown to occur *in vitro* in HIV-infected cells to the extent that HIV replication is inhibited (Kahlon et al., 1991). Kahlon demonstrated that HIV-induced cytopathicity in two human cell lines (CEM-SS cells and MT-2 cells) was significantly reduced in the presence of acemannan. The concentration of acemannan required to induce a 50% reduction in cytotoxicity was $45 \mu\text{g ml}^{-1}$. Other inhibitors of glycosylation, such as deoxymannojirimycin and swainsonine, also inhibited HIV cytopathic effects. However, unlike these compounds, acemannan was non-toxic at concentrations up to $1000 \mu\text{g ml}^{-1}$. Acemannan can therefore reduce HIV viral cytopathic effects at doses that are quite safe. Studies are in progress to see whether this holds true for FIV as well.

It has been suggested that acemannan can directly inhibit glycosylation of viral proteins based on the observation that the HIV surface glycoprotein

gp160 shows altered glycoforms when exposed to acemannan. Similar effects were also reported with Newcastle disease virus, a paramyxovirus (Kemp and Chinnah, 1989). The F (fusion) protein of paramyxoviruses share significant sequence homology with HIV gp41 transmembrane protein (Gallagher, 1987). Therefore, acemannan may similarly alter FIV glycosylation with a resulting reduction in viral function.

Adverse events

The only adverse events seen during the study were in four cats in Group 1 receiving acemannan by IV injection. It is believed that these events were due to a phenomenon occasionally seen when very large molecular weight compounds such as plasma expanders are injected by IV push. Although the exact mechanism has not been identified, this leads to rapid peripheral vasodilation and subsequent drop in blood pressure with resultant syncope, immediately followed by a physiologic response characterized by tachypnea, tachycardia and peripheral vasoconstriction. Recovery is usually rapid and uneventful. It is not surprising that a large molecular weight compound such as acemannan could cause such a reaction when administered IV as a bolus.

This phenomenon can be eliminated by mixing acemannan in 100–150 ml saline and administering by IV drip over a period of 15–30 min. No further incidents were observed when this treatment regimen was followed.

Survival

One goal of this pilot study was to compare various routes of acemannan administration for efficacy and for possible adverse effects. The results presented here suggest there is little difference between IV, SQ and PO routes in terms of efficacy. No statistically significant differences were seen in either clinical scores or laboratory values when treatment groups were compared.

Similarly, there was no difference in long-term survival noted among the three treatment groups. The survival curves do suggest, though, that IV administration may provide an advantage in the short term by enhancing survival within the first year of treatment. While this difference was not statistically significant, a larger study population may reveal more significant increases in survival by the IV route. For the present, it appears that the SQ route provides ease of administration while providing a survival rate nearly equal to that obtained in the IV group.

No statistically significant difference was found in survival of cats that entered with lymphopenia, compared with animals with normal lymphocyte counts. Six of the seven cats with Stage 5 disease were lymphopenic, two are still living (6+ and 11+ months).

Of the 36 cats still living, 23 (64%) have survived longer than 1 year after

initiation of treatment with acemannan. All but one were Stage 4 or Stage 5. Expected survival for Stage 5 (AIDS) cats is 1–6 months while survival of Stage 4 (ARC) cats ranges from months to 1 year (Ishida and Tomoda, 1990). Historical data on naturally infected cats with staged disease are very limited. Nevertheless, acemannan's effect on survival of FIV-infected cats seems encouraging.

CONCLUSIONS

Though adverse events were noted in animals treated by IV injection, these were readily explained as a physiologic response to acemannan's large molecular size. This response was easily eliminated by altering the method of administration.

No evidence of toxicity has been noted in any of the 49 animals treated in this study, 23 of which have been treated for more than 1 year.

Acemannan treatment resulted in a reduction of sepsis in FIV-infected cats and was associated with decreased neutrophil counts. Lymphocyte counts increased as a result of acemannan therapy. This suggests that acemannan may be useful as an aid in treatment of the immunosuppression that gives rise to chronic infections in FIV-infected cats.

In addition, survival rates in acemannan-treated cats exceeded those observed in limited historical controls in FIV-infected populations.

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