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Plasma Erythropoietin Activity before and after Renal Homotransplantation in Humans

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Summary. Subject and Method. Plasma erythropoietin levels were determined by Keighley's method to study the changes in erythropoiesis before and after human renal homotransplantation. Results. Erythropoietin titres in pre-transplant patients were low, while they returned to normal after successful renal transplantation. In acute rejection they were significantly high. After its reversal normal levels of erythropoietin were obtained in accordance with a normalization of the graft functions. Reticulocyte counts paralleled with erythropoietin values. Conclusion. High levels of plasma erythropoietin contribute to the diagnosis of acute rejection. Normalization of the plasma erythropoietin levels after the acute rejection could be regarded as an indication for good function of the graft. The grafted kidneys seem to function in producing erythropoietin.

Key words: Erythropoietin, transplantation, rejection.

Introduction

Anaemia has been recognized as one of the outstanding manifestations of chronic renal failure. Studies on the pathogenesis of this anaemia revealed that it is strongly concerned with 1) the aetiology of the renal disease, 2) failure of renal excretory function, and 3) failure of renal endocrine function (6).

The kidney has two major endocrine systems. One is the renin-angiotensin system and the other is erythropoietin production, which has been considered as a regulator of erythropoiesis. Since the report of Jacobson (11) the kidney has been considered as a major erythropoietin producing organ. This fact was also demonstrated by perfusion experiments on the isolated kidney by Kuratowska (14). It is now known that erythropoietin levels in plasma are low or undetectable in the terminal stage of chronic renal failure (8).

Since the 1950s human renal homotransplantation has been introduced in the treatment of chronic renal failure. Progress in this field has been made by immunosuppressive treatment and histocompatibility matching. Now there are long-surviving renal transplants. In such successful renal transplantation recovery from anaemia has been observed (3).

In this paper erythropoietin levels were determined before and after renal homotransplantation in order to know the changes of erythropoiesis.

Methods

Eight patients, who underwent renal homotransplantation for chronic renal failure at the Department of Urology, the University of Tokyo hospital, were examined. The original diseases were chronic nephritis and renal tuberculosis (Table 1.). All patients received immunosuppressive agents, such as azathioprine and prednisolone. In some patients actinomycin-D was administered. Local X-ray irradiation to the grafted kidney was employed during rejection crises.

Samples of venous blood were periodically obtained and stored at -20 C for subsequent assay. Plasma erythropoietin levels were assayed by erythrocyte radioiron uptake in polycythaemic mice according to the method described by Keighley (12).

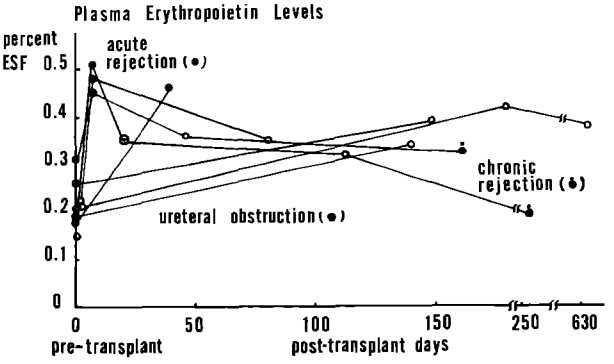
Table 1. Human renal homotransplantation performed in our deparment from Jan. 1966 to Jan. 1970

No.	Name	Age	Sex	Original disease	Donor	Survival
2	M.H.	21	M	Renal Tbc	Unrelated	2y 7m
3	T.S.	20	M	Chr. nephritis	Father	5m
4	Y.T.	26	M	Chr. nephritis	1) Father 2) Unrelated	29 days
5	S.C.	19	F	Chr. nephritis	Unrelated	11m
6	B.W.	33	M	Chr. nephritis	Unrelated	2y 1m
7	T.K.	26	M	Chr. nephritis	Mother	5m
8	H.N.	33	M	Chr. nephritis	Unrelated	9m
9	B.I.	35	M	Chr. nephritis	Brother	5y 3m

Polycythaemia was induced in female dd-d strain mice weighing 20 to 25 g by intermittent exposure to 0.5 atmosphere. Two weeks were required to make the mice polycythaemic by daily intermittent hypoxic stimulation for 12 hrs. The animals were then returned to ambient atmosphere (0 of assay period.). By this procedure the haematocrit of the mice rose to over 60% in most cases. Mice under 55% of haematocrit were not used. On the fifth day 5 mice received injection of 0.5 uC Fe59 citrate (Specific activity: 15 mC/mg. Daiichi Pure Chemical Corp. Tokyo) intravenously via the tail vein. On the seventh day they were killed by cardiac puncture. Radioactivity of the blood was determined in a scintillation counter (JDC 6. Type Aloka). Percentage of red cell iron incorporation was calculated by assuming an average blood volume 7.0 ml per 100 grams body weight.

Each assay showed the mean red cell iron incorporation in 5 test mice. Fe59-uptake in polycythaemic assay mice receiving normal plasma is 0.395 $\pm$ 0.0297 (mean $\pm$ standard deviation) %.

For the measurement of endogenous creatinine clearance (Ccr) determination of serum and urine creatinine was made. Para-aminohippurate clearances (CPAH) were also made by constant perfusion technique. The blood urea nitrogen was determined with a autoanalyzer (Technicon Instr. Corp. N.Y., U.S.A.) [2] All routine haematological studies were performed with standard methods.



Results

The data for eight patients in this study are shown in Figs. 1 and 2.

Plasma erythropoietin concentrations before transplantation were low in spite of anaemia, the mean value was 0.215 ± 0.049%. After successful renal transplantation almost normal levels of plasma erythropoietin were observed, the mean value 0.380 ± 0.037%.

Plasma erythropoietin was estimated in four patients who experienced acute rejection. It was observed that in acute rejection plasma erythropoietin concentrations (mean value 0.475 ± 0.029%) were significantly higher than those of the pre-transplant and the successful renal transplant state (statical significant deference. $p < 0.01$).

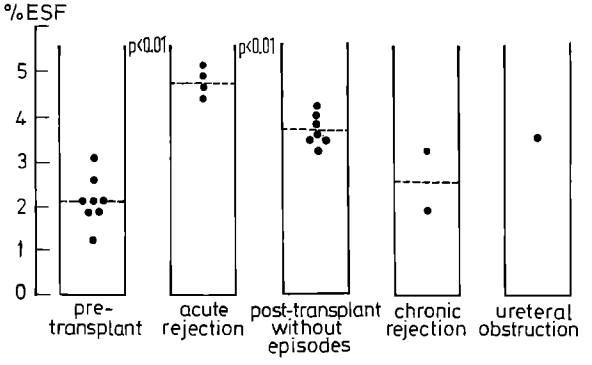


Fig. 2. Plasma erythropoietin levels before and after human renal homotransplantation

In chronic rejection as seen in cases No. 3. and 5., the plasma erythropoietin values were variable 0.32 and 0.19%).

Fig. 3 illustrates the relationship between haemoglobin, red blood cells, reticulocytes in the peripheral blood and the plasma erythropoietin values during the post-operative course. There was no correlation between haemoglobin, red blood cells, and plasma erythropoietin concentrations. However, parallel with the increase of reticulocytes, the erythropoietin values continued to rise, so that the correlation between them was found.

Concerning the renal function, including the levels of blood urea nitrogen, serum creatinine, Ccr, and CPAH, and the plasma erythropoietin values, there was significance of differences between the two groups with acute rejection and without episodes. After the reversal of acute rejection the plasma erythropoietin titres became normal in accordance with the normalization of renal functions (Fig. 4.).

Fig. 5 shows the renal function immediately after the transplant and at the peak of rejection attacks. At the rejection crises Ccr and CPAH showed a marked decrease.

Clinical signs, symptoms, and laboratory data were chronologically compared with the plasma erythropoietin values in four cases, No. 3, 5, 6, and 7 (Fig. 6.). These patients experienced acute rejection attacks, which were reversed by immunosuppressive therapy. Also two patients, No. 3 and 5, experienced chronic rejection crises, which could not be reversed by any means.

In the pre-transplant state these patients under-

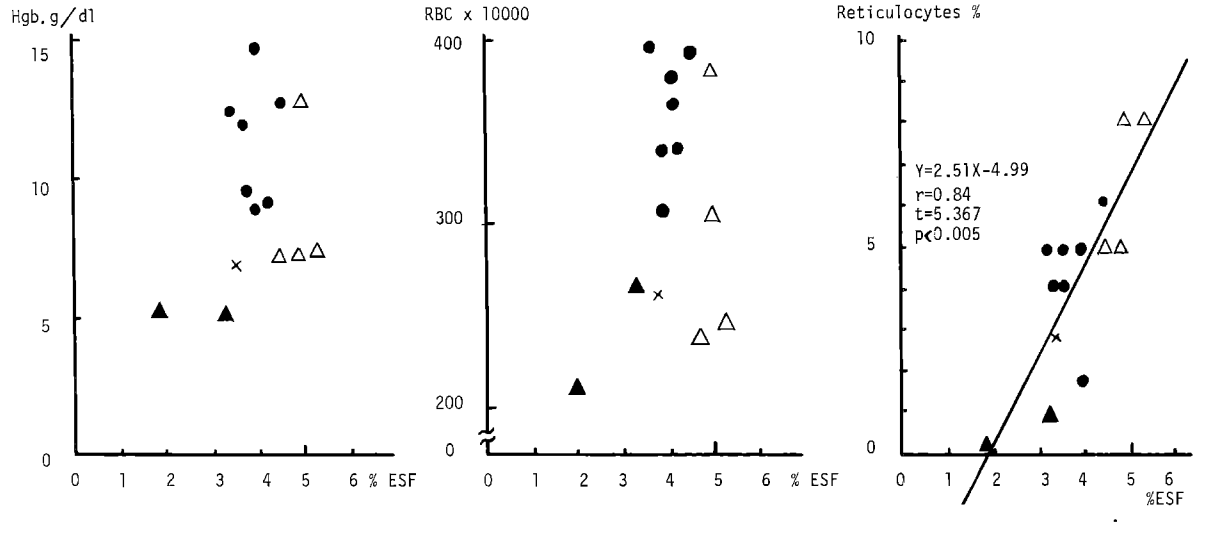


Fig. 3. Relationship between haemoglobin, red blood cells, reticulocytes in the peripheral blood and the plasma erythropoietin values during the postoperative course. The correlation was found between peripheral reticulocytes numbers and the plasma erythropoietin values.
△ = acute rejection. ● = without episodes. ▲ = chronic rejection. × = ureteral obstruction

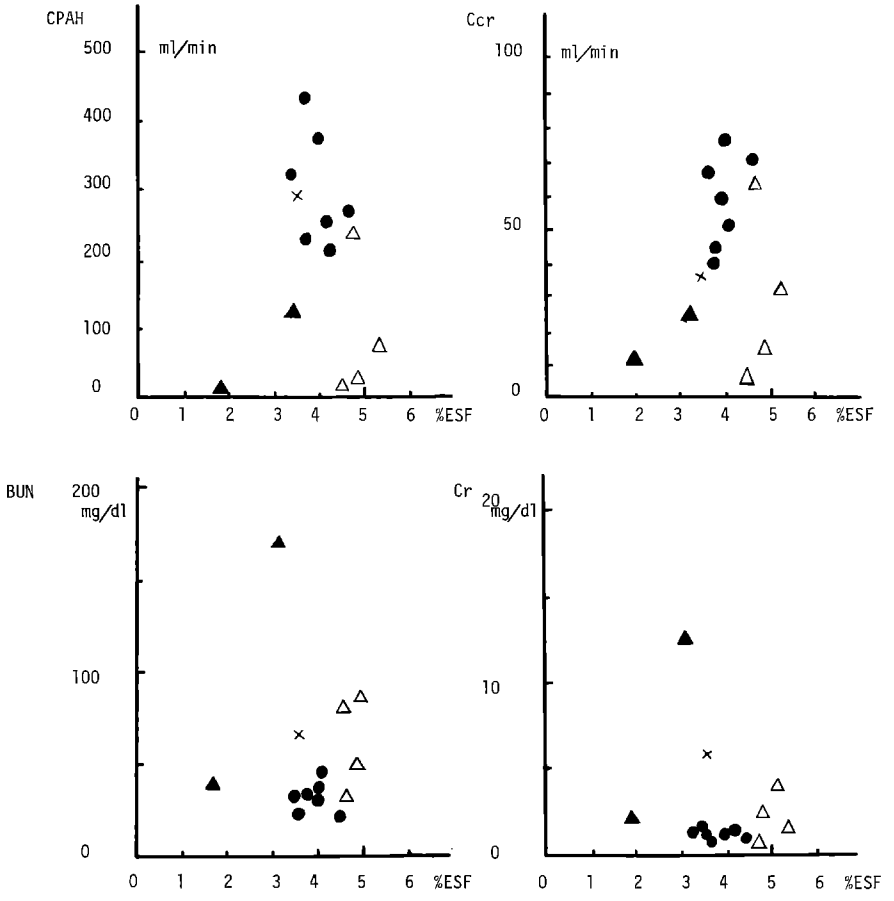


Fig. 4. Relationship between renal function and the plasma erythropoietin values during the postoperative course. There were statical differences between two groups: the acute rejection group and no episodes group. Statistical analysis was done by the discriminant function. In each case Fs was larger than Fo. p 0.01.

△= acute rejection. ●= without episodes. ▲= chronic rejection. ×= ureteral obstruction

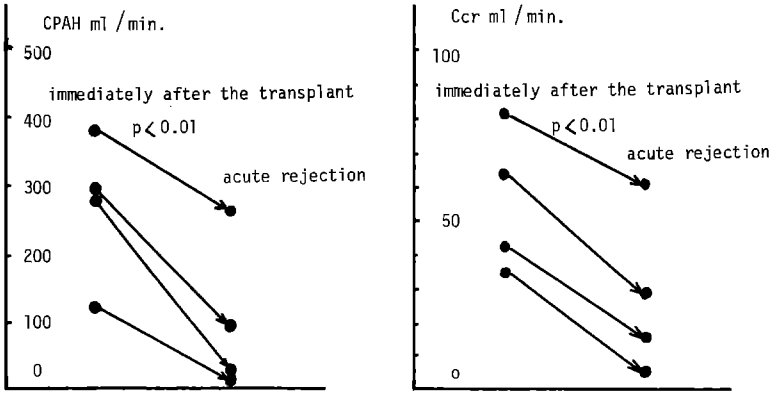


Fig. 5. Renal function immediately after the transplant and at the peak of acute rejection attacks. At the rejection crises Ccr and CPAH showed a marked decrease

went haemodialysis. However, generalized oedema and anorexia were found in cases No. 3 and 5. Daily urinary output was less than 600 ml. The specific gravidity of the urine was low, isosthenuric. Moderate proteinuria was also observed. The numbers of the peripheral leukocytes were not increased. In spite of frequent haemodialysis the levels of blood urea nitrogen and creatinine remained rather high. One week after the transplant these patients experienced severe acute rejection, characterized by general aedema, anorexia, elevated temperature, reduction of the urinary output, and reduction of renal function. At this time leukocytosis, elevation of blood urea nitrogen, creatinine, and reduced function of the grafted kidneys were found. However, plasma erythropoietin values were significantly high at the peak of acute rejection. The rejection reactions were reversed by increasing the doses of immunosuppressive drugs. 20 days after the transplantation ureteral obstruction, which was demonstrated by exploratory operation, occurred in the patient, No. 3. At this time the erythropoietin titre was 0.35%. Compared with acute rejection, there was no rise in the erythropoietin values. After operation the titre became almost normal. After the rejection attacks these patients showed almost normal renal functions. The erythropoietin titres went down nearly normal.

Two patients, No. 3 and 5, had chronic rejection. Their renal function deteriorated, but there were no signs and symptoms seen at the acute rejection attacks. At this time plasma erythropoietin values were variable (0.32 and 0.19).

Discussion

In the uraemic state plasma erythropoietin levels are reported to be low or undetectable by many investigators (8). In a larger series Abbrecht

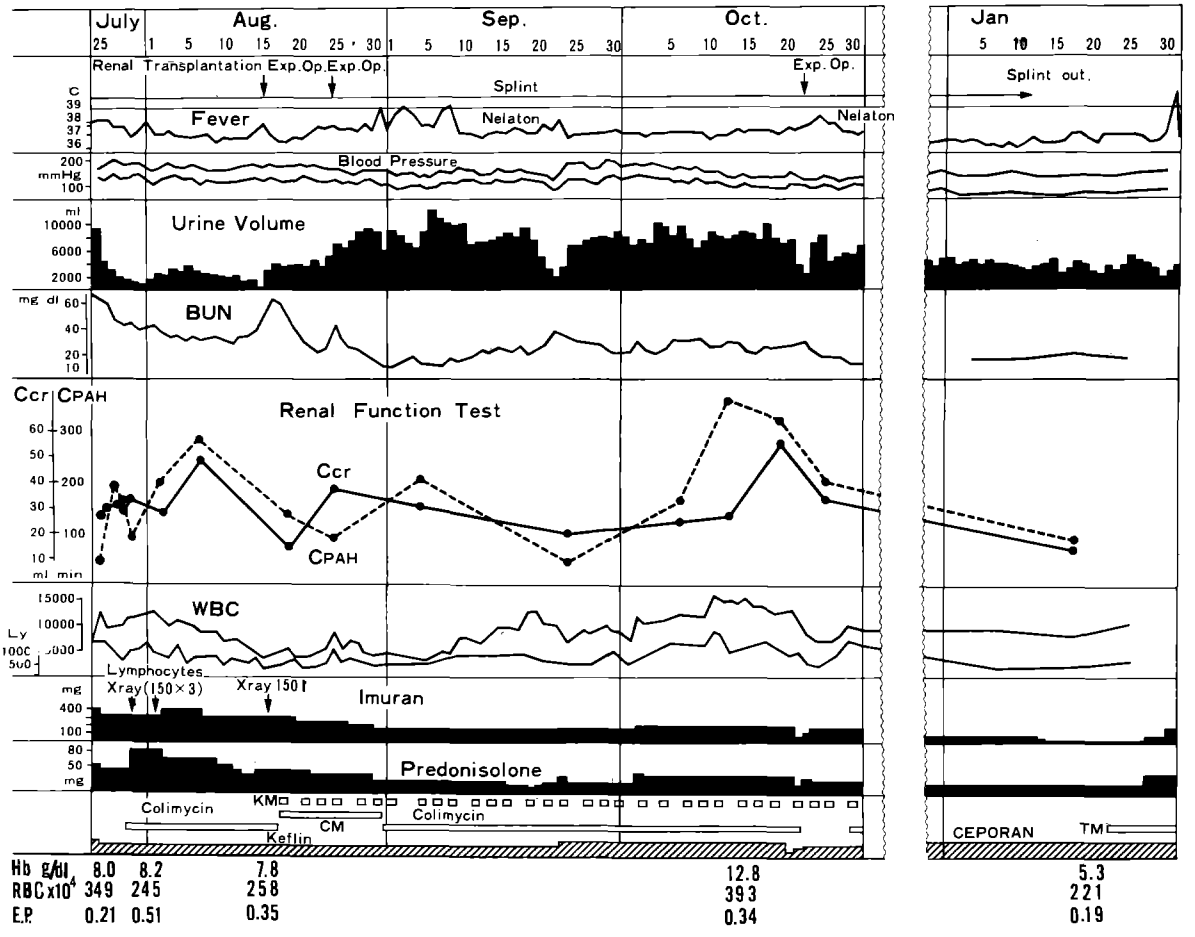


Fig. 6. The postoperative course in the patient No. 5. In the acute rejection the plasma erythropoietin was high, while it was almost normal when the ureteral obstruction was found. In the chronic rejection it went down low

and Green (1) noted little erythropoietin activity before renal homotransplantation. Pre-transplant plasma erythropoietin values in this study were low, which was in agreement with the data in the literature. This is supposed to be due to damage to the kidneys, which can not produce enough erythropoietin to stimulate erythropoiesis.

Some investigators (7) mentioned the presence of erythropoietin inhibitor in plasma, especially in uraemia. However, other investigators (4) were unable to detect erythropoietin inhibitor. It is presently unknown whether erythropoietin inhibitor plays a major role in the pathogenesis of renal anaemia.

At the peak of the acute rejection erythropoietin levels were significantly high, compared with the post-transplant values. Murphy (15) reported the same results in acute rejections. After the reversal of acute rejection the erythropoietin levels returned to normal.

During acute rejection impairment of renal function, especially glomerular filtration rate and renal blood flow, occurs. Histologically, as interstitial round cell infiltration develops, the interstitial tissue becomes oedematous. Mural or occluding thrombi may be found in many small veins and massive aggregates of platelets may develop in the glomerular capillaries (3).

In this study Ccr and CPAH showed a marked decrease at the peak of acute rejection crises, compared with the values immediately after the transplantation. It is supposed that a fall in renal blood flow, especially renal cortical flow, can be observed in acute rejection attacks, and the grafts receive less oxygen, becoming hypoxic. It is said that the kidneys have the capacity to produce erythropoietin in response to various forms of hypoxia (11). This is one of the factors which contribute to maintain the high titres of erythropoietin at the peak of acute rejection crises. Therefore, it is considered that the high levels of erythropoietin would be an aid to diagnose the acute rejection reactions.

Kountz (13) studied the haemodynamic situation by direct catheterization of renal artery and vein during the periods of acute rejection crises and showed that the slight fall in glomerular filtration rate and CPAH was not due to changes in renal blood flow. This discrepancy could be explained by more rapid transit of the blood through the kidney. Comparative studies of glomerular filtration rate and renal blood flow suggest that the early change is a fall in effective renal plasma flow, especially of cortical blood flow. Angiography during rejection crises may show diffuse occlusion of cortical arteries, but normal medullary vessels. According Hamburger (10) it is inconstant in reversible crises. In this study the graft functions; the blood urea nitrogen, creatinine, Ccr and CPAH, did not correlate with the plasma erythropoietin titres as a whole during the post-operative days. However, the statistical differences between renal function and the plasma erythropoietin values were demonstrated between two groups (with acute rejection and without episodes). So, a normalization of the plasma erythropoietin levels after the acute rejection could be regarded as an indication for a good function of the grafts.

In chronic rejection disturbances of renal function take place gradually. If late rejection is not overcome, irreversible changes would occur in the production of erythropoietin. On the contrary, if late rejection is reversed, the grafted kidney could produce erythropoietin. Therefore, variable levels of the plasma erythropoietin were observed in the rejection. Gra (9) reported that chronic rejection was associated with increased erythropoietin activity. In his case it is supposed that kidney function was relatively well maintained and could produce erythropoietin, even through gradual damage to the kidney was noted.

The kidney is now believed to be the major site of production of erythropoietin. There are various stimuli to the production. Renal hypoxia is one of the stimuli among them. In acute rejection crises the grafted kidney became hypoxic, which was considered to be a potent stimulus to the production of erythropoietin. Denny (5) assayed plasma erythropoietin from one week to ten months after transplantation of the kidneys in ten patients. Five out of ten patients had erythropoietin elevation within one to forty days after transplantation. In this study the plasma erythropoietin values were high at the peak of acute rejection. After the rejection crises were reversed the grafted kidneys received more oxygen, so that reduced stimulation to its production was anticipated. As anticipated normal levels of the plasma erythropoietin was obtained after the reversal. In chronic rejection variable levels of erythropoietin were demonstrated. These varying titres of the plasma erythropoietin suggest that the grafted kidneys, responding to the various stimuli. seem to contribute to the production of erythropoietin.

Numbers of reticulocytes in the peripheral blood had a close parallelism with the plasma erythropoietin concentrations after the renal transplantation. An increase in the number of reticulocytes in the circulation is the surest index of accelerated erythropoiesis (16). Therefore it is considered that a rise of reticulocyte count in the peripheral blood seems to show the capability of the erythropoiesis to respond to erythropoietin.

There was no relationship between haemoglobin, red blood cells and the plasma erythropoietin concentrations as a whole. However, in successful renal transplants plasma erythropoietin levels and renal functions were almost normal. Therfore, the bone marrow can respond enough to the stimulation of erythropoietin, which leads to the recovery from anaemia. At the peak of acute rejection renal functions deteriorated. Plasma erythropoietin concentrations were so high that recovery of anaemia

was expected. However, anaemia was found in three out of four patients who experienced acute rejection crises occurred within two weeks after renal transplantation. Therefore, it is considered that the bone marrow can not respond enough to the stimulation of erythropoietin in acute rejection crises.

In chronic rejection deterioration of renal function was found, while the plasma erythropoietin values showed variable. In such cases anaemia was noted.

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