Medical Sciences

Intramyocardial and Extramyocardial Autologous Endothelial Cell Transplantation for Treatment of Ischemic Cardiomyopathy (Experimental Investigation)

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ABSTRACT. Regional myocardial perfusion and function may improve after cell transplantation. The objective of the study was to investigate the feasibility and efficacy of autologous endothelial cell implantation into myocardium or transplantation onto myocardium using a fibrin matrix.

An ameroid constrictor was placed on the circumflex artery of 48 adult sheep to induce ischemic cardiomyopathy. Four weeks later, the animals were divided into the following six groups (n=8 in each group): cardiomyoplasty alone without cell transplantation (CMP), cardiomyoplasty with EC added to a fibrin platform and placed between the myocardium and latissimus dorsi muscle (CMP+EC), autologous EC transplantation (AEC), saline injection with added denaturated cells (SAL), fibrin sealant injection without EC (FS) or a control (CON) group. Eight weeks after treatment the left ventricular function was investigated and the animals were sacrificed. Myocardial blood flow and capillarization were evaluated.

Eight weeks after injection (AEC, SAL, FS groups), or cardiomyoplasty (CMP, CMP+EC groups) or control (CON) groups, ventricular function was markedly improved in the CMP, CMP+EC and AEC groups (p<0.05 versus baseline, SAL, FS and CON), but had deteriorated in the SAL, FS and CON groups (p<0.05 versus baseline). Myocardial blood flow was also deteriorated after ameroid constrictor placement, but 8 weeks later was increased in the EC-group.

Myocardial blood flow was also deteriorated after ameroid constrictor placement (p<0.05 in all groups vs. baseline), but 8 weeks later was increased in the EC and CMP+EC groups (p<0.05 vs. before treatment). In the CMP, SAL, and CON groups myocardial blood flow continued to deteriorate for the next 8 weeks. In the FS group myocardial blood flow had a tendency to increase after treatment, but statistically non-significant. Histology and electron microscopy revealed extensive neovascularization and improved myocardial appearance after endothelial cell implantation into myocardium and cell transplantation between myocardium and latissimus dorsi muscle. In the other groups capillary density was considerably deteriorated.

These results suggest that intramyocardial transplantation of autologous EC within a fibrin matrix and application of EC to the myocardium during cardiomyoplasty enhances neovascularization, increases myocardial blood flow, and improves the left ventricular function.

Key words: intramyocardial transplantation, ischemic cardiomyopathy.
Introduction

Today, hundreds of thousands of patients who have been diagnosed with heart failure or either pre-end or end stage ischemic cardiomyopathy are not helped by the available medical and surgical treatment interventions. Despite the current treatment options of coronary artery bypass surgery and angioplasty with or without stent implantation, this patient population, which includes those individuals who have severe diffuse coronary artery disease or small vessel disease, continues to suffer severe anginal pain with undiminished high risk to viable myocardial cells. Two unavoidable processes lead these patients to their inevitable demise: aggravation of atherosclerotic ischemic coronary artery disease and the inability of the ventricles in a highly ischemic heart to support adequate hemodynamics. Heart transplantation is an answer for only a few due to a lack of available donor organs.

Thus the challenge for present day cardiologists is how to treat patients with advanced ischemic cardiomyopathy, who have a limited chance at heart transplantation or who have refused other surgical treatment options. One of the most promising new approaches for treatment in such patients is neovascularization of ischemic tissue or the border zones through local stimulation of angiogenesis [1, 2]. It has been shown [2-4] that ischemic tissue injury causes the release of special endogenous biochemical agents, including growth factors, which stimulate angiogenesis through collateralization of the available vessels. Unfortunately the process of collateralization of the available capillaries and arteries requires more time than does vascular narrowing or occlusion. This angiogenic response is often inadequate to prevent clinical manifestation of ischemia. Therefore, processes needed to be found, which accelerated and artificially enhanced the development of the new vessels before irreversible tissue damage took place.

The concept of promoting new vessel growth using growth factor therapy is intriguing. This approach differs from previous revascularization techniques and may provide a means to treat patients who are not candidates for current standard treatment options. Direct application of vascular growth factors or DNA encoding for such factors have been tried [5, 6], but it was difficult to prove whether these agents remained at the target long enough to be specifically active. The demonstration of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) to elicit an angiogenic response via their own endothelial receptors has led to subsequent experimental and clinical investigations to study their use in myocardial revascularization. However clinical results have ranged from merely questionable to speculative [7-12].

Another area of research involves utilization of mature ECs and EC-precursor cells [13-15]. In many ways, the magnitude of risk is equal for both harvesting bone marrow and for mobilizing and collecting EC-precursors from peripheral blood vessels. It is unknown, however, whether heterotopic EC transplantation will produce enough vascular cell proliferation to form a functional capillary network in order to revascularize ischemic tissue.

It also was necessary to devise a method for the prolonged release of endothelial cells into the ischemic tissue. Studies from several laboratories [16, 17], including our own [18], have shown that fibrin can be used as a carrier for the application and prolonged delivery of agents. A fibrin network is critical for effective wound healing, and is biodegradable through routine tissue fibrinolysis. Since the fibrin sealant is slowly lysed, it can serve as a vehicle to deliver agents that may act to help heal wounds [19-24] to promote new vessel growth [16, 25] or to store and slowly release any therapeutic agent [19]. In preliminary investigations, we used fibrin sealant to deliver aprotinin and pyrrolostatin to ischemic skeletal muscle in order to accelerate local revascularization [26, 27]. We also used the fibrin platform to endothelialize vascular grafts and cell transplantation [28, 29].

We have hypothesized that a fibrin platform could be used to support the viability of applied or transplanted EC, direct the morpho-functional process of capillary formation, and accelerate compensatory myocardial revascularization in ischemic myocardium. In this chapter for the first time feasibility and efficacy of autologous mature EC application or transplantation in a fibrin matrix is described in a large animal model. The strategy of mature EC transplantation used in this study contrasts with approaches used before, including GF therapy, bone marrow cell transplantation and ex vivo expanded EC progenitor transplantation.

Materials and Methods

An ameroid constrictor was placed on the circumflex artery of 48 adult sheep to induce ischemic cardiomyopathy. Four weeks later, the animals were divided into the following six groups (n=8 in each group): cardiomyoplasty alone without cell transplantation (CMP), cardiomyoplasty with EC added to a fibrin platform and placed between the myocardium and latissimus dorsi muscle (CMP+EC), autologous EC transplantation (AEC), saline injection with added denaturated cells (SAL), fibrin sealant injection without EC (FS) or a control (CON) group. Eight weeks after treatment, left ventricular function was investigated and animals were sacrificed. Myocardial blood flow and capillarization were evaluated.

Preparation of autologous endothelial cells.

Endothelial cells were cultivated from the endothelium of the jugular veins of sheep using standard [30]. After removal, the vessels were immersed in Hanks solution supplemented with penicillin/streptomycin and fungizon...
and immediately transported to the cell culture laboratory, where they were opened longitudinally. The vein was placed on a Petri dish, irrigated with a few drops of 0.2% solution of collagenase (type IA, Sigma), and incubated at 37°C for 15 minutes. Next, it was washed with a medium containing serum and centrifuged at 600 rpm for 5 min. The pellet was resuspended in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with serum, and placed into a T25 culture flask.

Preparation of fibrinogen. Standard cryoprecipitate technique was used to prepare autologous fibrinogen from sheep plasma collected one week before EC seeding. Whole blood was collected in 50cc polypropylene centrifuge tubes (Fischer Scientific, Pittsburgh, PA) containing citrate-phosphate dextrose anticoagulant solution and centrifuged at 4°C at 1,750 rpm for 20 min. The plasma was then separated from red cells, frozen, and stored at –20°C for 18 h to 24 h before further processing. The frozen plasma was thawed in a 4°C cold room for 4 h and centrifuged at 2°C at 2,500 rpm for 40 min, then drained, leaving 3–4 ml of concentrated fibrinogen, approximately 20 mg/ml.

Sources of thrombin. In all experiments bovine thrombin (Johnson and Johnson Medical, Inc. Arlington, TX) was used. Lyophilized thrombin was solubilized in complete cell culture medium and diluted to an activity 3 U/ml.

Experimental animals. The animals were cared for according to the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” published by the National Institutes of Health.

1) General Anesthesia and Antibiotic Therapy

All animals underwent general anesthesia for each surgical procedure. The animals were premedicated with Valium (Elkins-Sinn, Cherry Hill, NJ; 5 mg/kg IV) and anesthetized with sodium Pentothal (Abbott Laboratories, North Chicago, IL; 20-25 mg/kg IV). They were then intubated, placed on a Draeger (North American Draeger, Telford, PA) ventilator, and maintained on Halothane gas anesthesia at all times to reduce the potential for infection. The animal’s tongue. Strict sterile technique was followed heart rate were monitored via a pulse oximeter placed on the animal’s tongue. The electrical stimulation protocol was begun 14 days after LDM mobilization and heart wrap with a cardiosynchronization ratio of 1:2, with an amplitude of 5 V and a frequency of 10 Hz. Stimulation was begun with single impulses for two weeks, followed by double im-
pulses (two weeks), triple impulses (two weeks), and fin-
ally two months after CMP, trains of impulses (30 Hz, 
burst of six impulses, pulse width of 210 msec) synchro-
nized 1:1 with the cardiac cycle (the number of impulses 
and frequency changed) were given.

**Histology**

1. **Biopsies** for light microscopy, immunohistochemistry 
and transmission electron microscopy were taken from 
the LDM before and after subtotal mobilization on the days 
designated in the experimental protocol. At the termina-
tion of experiments, the heart/muscle complex after CMP 
was carefully excised. Samples from the different areas 
(proximal, middle and distal) of the LDM, and the apical, 
middle and base regions of the heart were taken for analy-
sis. Samples (3x4 mm) for light microscopy and immuno-
histochemistry were placed in 10% Formalin and taken to 
the hospital’s pathology department for embedding and 
sectioning. Samples (3 x 4 mm) for transmission electron microscopy (TEM) were placed in Karnovsky’s fixative.

2. **Light microscopy.** For histological examination, samples 
were taken from each area and fixed as described above. 
Transverse sections were made for conventional histo-
logical (Hematoxylin & Eosin) staining and for subse-
quent evaluation. Multiple slides were made of each bi-
opsy sample. Histological data were submitted for inter-
pretation to an independent observer. Particular atten-
tion was paid to evidence of muscle regeneration, thick-
ness and composition of the reparative response, the 
density of neovascularization, the presence of large bore 
vessels, and margination of leukocytes.

3. **Transmission electron microscopy (TEM).** Biopsies of 
the LDM (approximately 3x4 mm) for transmission elec-
tron microscopy were placed into Karnovsky’s fixative 
(2% formaldehyde, 2.5% glutaraldehyde in 0.1 M Sodium 
Cacodylate buffer, pH 7.2) and then sliced into smaller 
(1-2 mm) pieces. The biopsies were postfixes in a 1% 
Osmium Tetroxide, dehydrated through a series of graded 
alcohols and acetone, and embedded in Spurr resin. Pre-
liminary thick (1 mM) sections were cut and stained with 
0.1% Toluidine blue. Longitudinal areas of muscle were 
selected for ultra-thin sectioning; thin sectioning (60-90 
nm) was done with a Reichert Ultra-Cut microtome. The 
thin sections were stained with 5% Uranyl Acetate and 
Reynolds’s Lead stain (3.5% lead citrate, 2% lead nitrate). 
Examination and photography of the thin sections were 
done with a Philips 400T transmission electron micros-
cope at an accelerating voltage of 60 kV on Kodak SO-
163 image film. Five electron micrographs of adjacent ar-
eas in each of 2 regions were taken on each biopsy at a 
magnification of 6000x. A 2800x magnification of the two 
regions was also included as an overview. Special atten-
tion was paid to the morphology of capillaries, pericytes 
and smooth muscle cells.

4. **Conventional indirect immunofluorescent en-face staining (immunohistochemistry [IH]).** In order to as-
sess angiogenesis, conventional indirect immunope-
roxidase staining was used after fixation and proteolytic 
predigestion of formalin-fixed tissue followed by incuba-
tion with von Willebrand Factors (vWF) as angiogenic markers. This analysis yielded information as to overall 
angiogenesis and vascularization in the ABG, as well as the adjacent skeletal muscle and myocardium. The de-
gree of vascularization was evaluated by counting the 
number of vessels per unit area.

**Blood Flow Analysis**

**[Fluorescent Microspheres]**

After completing the thoracotomy, one temporary catheter was placed into the femoral artery and one in 
the left atrial appendage. A temporary occluder was placed 
on a previously isolated coronary artery, and blood flow 
was occluded for 3 minutes during which time 15 million 
fluorescent microspheres (15 μm) were injected into the 
left atrium. A reference blood sample was simultaneously 
withdrawn from the femoral artery at a fixed rate (Harvard 
Apparatus Withdrawal/Infusion Pump). Blood flow to the 
area at risk through the innate intramyocardial collateral 
system was able to be determined. Both temporary cath-
eters and the temporary occluder were removed, and 
blood flow restored. On day 60, catheters were again 
placed into the left atrium and femoral artery. In animals 
with cardiomyoplasties (series IV), an additional catheter 
was placed in the thoracodorsalis artery as well. Fifteen 
million fluorescent microspheres of a different color were 
 injected into the left atrium, and a reference blood sample 
taken. Blood flow representing the flow delivered by the 
native intracoronary and the extramyocardial collaterals 
from the LDM were able to be determined. In animals 
from Series IV, seven and a half million microspheres of a 
third fluorescence were then injected directly into the 
thoracodorsalis artery to determine collateral blood flow 
from the LDM. From this injection the ratio of microspheres found in the heart in relation to those found 
in the LDM could be determined. After euthanization, 
the heart/muscle complex was removed. Samples of the 
heart and LDM were taken for the blood flow.

**Data Analysis**

Histological data was submitted to one of the 
hospital’s pathologists for interpretation and evaluation. 
Hemodynamic data and capillary numbers were collected 
and tabulated. Values are reported as mean plus or minus 
standard deviation of the mean. Data was compared using 
an ANOVA and a subsequent Student Newman-Keuls 
t-test if applicable. A $p$ value of less than 0.05 was con-
sidered statistically significant.
Results

I. Animals without cardiomyoplasty (AEC, SAL and CON groups)

1. Assessment of left ventricular function. Baseline LVEF was 0.68 ± 0.03, LVESV was 26.4 ± 6.1 ml, and LVEDV 53.4 ± 10.4 ml. At four weeks after ameroid placement, LVEF decreased to 0.49 ± 0.05 (p=0.0000000004), LVEDV increased to 68.1 ± 9.9 ml (p=0.0019) and LVESV to 42.4 ± 7.5 ml (p=0.000006).

Two months after treatment, in animals subjected to EC transplantation (AEC group), LVEF increased to 0.56 ± 0.04 (p=0.029 vs. four weeks after ameroid placement). In the control group (no treatment), mean LVEF decreased to 0.40 ± 0.09 (p=0.013 vs. EC groups); and in SAL group EF decreased to 0.39 ± 0.05 (p=0.0017 vs. AEC group).

LVEDV increased in all three groups: to 81.3 ± 9.9 ml in the CON group and to 76.1 ± 11.6 ml in the SAL group. Although we observed an increase in LVEDV in the EC group to 60.4 ± 2.7 ml, this parameter was significantly lower than in the control groups (p=0.006 vs. CON group).

LVESV in control animals increased to 51.1 ± 7.5 ml and to 53.8 ± 6.2 ml in the SAL group. However, LVESV was significantly lower after EC transplantation (30.1 ± 4.1, p=0.002 vs. CON group and p=0.0007 vs. SAL group).

2. Myocardial Blood Flow. Myocardial blood flow (MBF) before placement of the ameroid constrictor was 0.59 ± 0.05 ml/min/gm. One month after ameroid constrictor placement, blood flow in the ischemic part of the myocardium decreased to 0.41 ± 0.07 ml/min/gm (p=0.0000009). At the time of sacrifice, MBF decreased to 0.15 ± 0.03 in the CON group (p=0.00001 vs. four weeks) and to 0.18 ± 0.05 in the SAL group (p=0.0006 vs. four weeks). In AEC animals, MBF increased to 0.66 ± 0.10 ml/min/gm (p=0.0009 vs. CON group and p=0.0001 vs. SAL group).

3. Immunostaining. In normal nonischemic myocardium, in all three groups, 5.6 ± 0.4% of the area was occupied by capillaries. Three months after ameroid constrictor placement, in the CON group this percentage decreased to 3.3 ± 0.3% (p=0.003 vs. baseline) and to 3.4 ± 0.6% in SAL group (p=0.009 vs. baseline). In the EC group, however, the area occupied by capillaries increased to 8.4 ± 1.3% (p=0.0004 vs. baseline, p=0.00027 vs. control group and p=0.00016 vs. SAL group).

4. Transmission Electron Microscopy. In both CON and SAL animals, the typical pattern of ischemic damage (cytoplasm folding and projection into the capillary lumen) was seen in the EC in ischemic vasculature. However, in the border areas of ischemic zone a few ECs appeared normal and had no signs of damage or injury. Perhaps these cells were able to give rise to new capillaries and arterioles. In contrast, tissue samples obtained from animals in the AEC group had EC that appeared normal with significantly enhanced vascularization and no signs of significant ischemic lesions.

II. Animals with Cardiomyoplasty (CMP group)

1. Assessment of left ventricular function

LVEF increased after cardiomyoplasty to 0.59 ± 0.03 (p<0.037 vs. 4 weeks after ameroid placement). LVESV was 28.0 ± 5.1 ml and LVEDV was 56.1 ± 4.7 ml (p<0.05 vs. SAL and CON groups but p>0.05 vs. the AEC group).

2. Myocardial Blood Flow

MBF was 0.60 ± 0.08 ml/min/gm. It was better than in the CON group (0.15 ± 0.03, p=0.0004) and in the SAL group (0.18 ± 0.05, p=0.008), but worse than in the AEC group (0.66 ± 0.10, p=0.077). Blood flow in the lattissimus dorsi muscle before mobilization was 0.36 ± 0.04 ml/min/gm and 0.15 ± 0.04 ml/min/gm after mobilization (p=0.0019). After EC application blood flow increased from 0.15 ± 0.04 to 0.41 ± 0.06 ml/min/gm (p=0.0003).

3. Immunostaining

The area occupied by capillaries increased from 5.6 ± 0.43% to 7.7 ± 0.8% (p=0.0027). It was considerably better than in the control series (p=0.00091) and SAL series (p=0.00058), but worse than in the AEC group (p=0.036).

4. Transmission electron microscopy

Transmission electron microscopy revealed new capillary formation in the fibrin glue between the myocardium and the LDM, and well-preserved capillaries in the ischemic part of the myocardium.

Discussion

Transplantation of mature cells has been proposed as a strategy for organ replacement or tissue repair (neomorphogenesis) for a variety of therapeutic needs, e.g., generation of bioengineered skin, blood vessels, liver, nerves, bone, and cartilage [31-34].

A crucial component of transplantation procedure is forming the three-dimensional biodegradable matrix in which neomorphogenic processes take place. These matrices are generally used for cell anchorage, guided migration, proliferation, and differentiation. The ones currently used in cardiovascular bioengineering are potentially immunogenic; they show toxic degradation and inflammatory reactions and act primarily as passive scaffolding for cell manipulation in culture or for implantation as a film.

Rather than a passive matrix, for EC transplantation, we needed a matrix that had two essential characteristics: morphogenically active to direct implanted cells to form vessel-like structures and an injectable liquid to polymerize and solidify after administration into target territory. Fibrin-based materials have these and many other characteristics beneficial for bioengineering a vascular bed.

We also needed to be able to generate a specific cellular signal that would direct the morphogenesis of implanted cells to form a neovascular network that would be connected to existing vessels. The idea of using fibrin as an angiogenesis substance either alone or with
the addition of proteins belongs to Fasol et al. [16], who demonstrated in a rat model that significant site-directed formation of new blood vessel structures could be induced by using a modified fibrin glue implant that contains the angiogenic growth factors HBFG-1.

In previous experiments we found that a fibrin-based sealant becomes vascularized [18, 35], thus capable of delivering some of the plasma proteins needed to perform some functions and extracellular matrix to anchor EC to the vessel wall [28]. We also demonstrated clinically in patients with PAD that VEGF and a fibrin-based sealant accelerated angiogenesis [36, 37].

The present study is the first to show that EC confined in a 3-dimensional fibrin matrix will form capillaries in ischemic myocardium. In microvascular ECs, we previously [38] found two morphogenetically distinct types of growth. Cultured in a two-dimensional matrix, ECs quickly form a cobblestone monolayer that has a density 2 – 2.5 fold higher than in controls, i.e., those cultivated on a single-plane tissue culture surfaces. We hypothesized that the ionic strength of the ratio of these components enabled the cells to “recognize” minor spatial changes in fibrin-meshwork architecture. Cultured in a three-dimensional matrix, ECs formed “true” capillaries while other vascular cells trapped in this matrix underwent apoptosis [39].

Because the direction in which capillaries form is generally determined by the orientation of the administered angiogenic mixture, we needed to ensure that the EC would grow in the desired direction. On the other hand, ischemic tissue itself produces many chemotactic factors that attract EC or EC-precursors and will support capillary growth, suggesting that plasma proteins can perform some of the functions of the extracellular matrix involved in anchoring EC to the myocardium.

Because it is well known that ischemia interrupts local circuit neurons, previous studies showing that fibrin enhances nerve regeneration [40, 41] point to another mechanism for improving LV function after EC/fibrin intramyocardial injection [42].

During our investigation we also showed that application of EC in biological glue when added to ischemic LDM increased blood flow to the muscle more than two fold, compared with non-treated mobilized muscle.

Application of glue and EC between the LDM and myocardium (during CMP or injection of EC directly into the ischemic myocardium) creates strong adhesions between the two layers, provides for capillary growth, and increases revascularization of the ischemic and nonischemic myocardium.

In the clinical setting our proposed strategy would require that the patient donate autologous EC and reagents for the fibrin matrix, thus also avoiding potential immunological rejection. As has been clearly demonstrated, transplantation of allogenic cells, no matter how freshly prepared or viable, provokes strong conflict with a spectrum of likely immunological and inflammatory events that lead to rejection [43]. Thus, autologous cell transplantation is a reasonable, if not mandatory, step, but this raises the problem of how to apply this laboratory technique clinically, namely, what source should be used for autologous EC.

Certainly, patients who have cardiovascular disease cannot be autologous donors of EC derived from jugular, cephalic or saphenous veins. Adipose tissue has been actively explored as an alternate source of EC because of its abundance in the body, easy access, and its composition of primarily two cell types, i.e., adipocytes and capillary EC. In culture, these EC demonstrate many of the functional and morphologic characteristics seen in EC in large vessels, hence, large quantities of adipose tissue could be removed with minimal risk to the patient and the inherent difference in density between adipocytes and capillary fragments means that they can be easily separated by simple centrifugation.

Once a human adipose tissue EC procurement protocol is developed, its use for high density seeding has to be validated by successive successful studies of culturing of EC derived from patients with cardiovascular diseases. One such study of harvesting adipose tissue EC by means of liposuction has already reported 100% success in 140 patients [44].

Finally we think that cell transplantation is more potent than GF therapy. Although it was previously demonstrated that intramyocardial transplantation of bone marrow cells induce neovascularization of ischemic tissue [13, 14], it remains to be proven that these cells indeed differentiate into mature EC and not into other types of cells including fibroblasts, osteoblasts, etc.

Recently Kawamoto et al. [15] demonstrated the therapeutic potential of ex vivo expanded endothelial progenitor cells in a small animal model. However, the safety of ex vivo culture expansion needs to be established.

Conclusions

Although endothelial cell transplantation induced capillary growth in the myocardium and improved circulation in the ischemic muscle in a sheep model, clinical trials are needed to investigate the efficacy of this intriguing technique. We suggest that this technique or a similar one that uses EC application (in the case of cardiomyoplasty) to accelerate the formation of compensatory collateral vasculature may be an answer to one of the most challenging issues in contemporary cardiology: how to treat small vessel pathology and disseminated lesions. Future approaches may consist of delivering a “cocktail” of angiogenic agents laced simultaneously with vasculogenic precursors and mature vascular cells in a temporally, spatially coordinated fashion.
ბარტყებრთა ხერხები და აღმოჩენილი პროცესები და მათი ტექნიკური სხეულური სტატისტიკური განიშვრები, სადასჭირებელია მაქსიმალური ულტრაუნდირული გავრცელების გამჭვირვალობის გამოყენებით.

საფარი ქალაქის დეპარტამენტიდან პირველი და მეორე წლის პირველი კარდიამოჭრილი გაუქმები მაქსიმალური ულტრაუნდირული გავრცელების გამჭვირვალობის გამოყენებით.

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D. ქართულ დღის გამოყენებით, მოცეფილობი, პირველი და მეორე წლის პირველი კარდიამოჭრილი გაუქმები მაქსიმალური ულტრაუნდირული გავრცელების გამჭვირვალობის გამოყენებით.

იგულისხმევით გამოყენებით, და მისი მხრივ ბიუჯეტის შესაძლებლობაში გავრცელების გამჭვირვალობის გამოყენებით.

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REFERENCES


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