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OVERVIEW

Kidney preservation in the next millenium

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Abstract For the past decades, severe hypothermia has represented the foundation of organ preservation in clinical transplantation. Beneficial as hypothermia has proven to be in preserving grafts from heart-beating donors, hypothermia does not seem to provide the window necessary for the prospective evaluation of organ function. With the increasing use of non-heart-beating donors, it is logical to propose that if organs are to be evaluated prospectively, it will be necessary to preserve them at warmer temperatures. Since both glomerular and tubular functions are inhibited at temperatures below 18 °C, such a goal will necessitate organ preservation at a temperature above 20 °C. The principle of preservation at warmer temperatures is not new, but with future developments and approaches, successful realization appears within reach. In this overview, a brief history of previous attempts at warm preservation, in the context of the current status of kidney preservation, is presented. Future developments and approaches, with the potential for prospective testing of the function and enhanced resistance to ischemic damage, will be discussed.

Key words Kidney preservation · Viability testing · Ischemia · Non-heart-beating donor · Heart-beating donor

Organ preservation and the organ shortage

The evolution of transplantation has made kidney allografting the preferred treatment for patients with end-stage renal failure. Transplantation provides improved quality of life and is more cost-effective than dialysis [33]. Currently, 1-year graft survival rates for cadaveric donor transplants are above 80% and 1-year patient survival rates 95% [100].

Offsetting this success is the increasing discrepancy between the availability of, and demand for, transplantable kidneys. Within the Eurotransplant region, procurement rates remained stable during 1996 and 1997, whereas the number of potential recipients on the waiting list increased by 4% [25], resulting in approximately 3,000 procedures compared to 11,000 patients awaiting transplantation [70].

A major factor contributing to the organ shortage is the detrimental effect of ischemia. Upon harvesting, the loss of the vascular circulation causes ischemia, with the concomitant depletion of oxygen and nutrients to the kidney. The ischemia, with the resulting loss of metabolic activity, initiates a cascade of cellular damage. This injury cascade leads to the breakdown of high-energy compounds necessary for cellular metabolism, a generalized loss of cellular integrity, and activation of degenerative enzymes. The result is the loss of all structural and functional components of the cell. While it has been proposed that the kidney can tolerate as much as 2 h of warm ischemia before the damage becomes so severe that it is irreversible and reperfusion with blood will not restore a life-sustaining function, transplanting an allograft with warm ischemic exposure of this magnitude is never considered clinically. Once a

kidney is removed, preservation techniques must preserve the organ sufficiently to ensure that the organ functions when transplanted and must preserve it over a long enough period to allow time for tissue typing, matching, and transportation.

Since the 1950s, hypothermia has been known to provide considerable protection against ischemic damage [14, 50, 85, 88]. The effectiveness of hypothermia can be explained by its ability to suppress the organ's metabolic rate approximately 12-fold by cooling to 4°–8°C. It was later discovered that inhibition of cell swelling and acidosis by a preservation solution in combination with hypothermia could further extend the preservation period, the so-called solution effect. There are two methods of hypothermic preservation in use today: cold static storage (CS) and continuous, cold machine preservation (MP). CS was developed using a crystalloid, intracellular-like solution. Much of the early work in CS was done by Collins et al. [26]. Belzer et al. successfully applied the principle of MP for kidney preservation [6]. MP involves the use of a machine to pump a cold perfusate through the organ at low pressure, simulating metabolism by supplying oxygen and nutrients and removing metabolic end products. The mostly widely used perfusate for MP, developed by Belzer's group, contains 5% hydroxylethyl starch (HES) as a colloid to prevent interstitial edema, gluconate to prevent cell edema, glutathione and a high-potassium concentration to prevent losses of these components from the intracellular compartment, and adenosine and phosphate to allow for maintenance of intracellular high-energy phosphate pools.

For more than 30 years, there has been an ongoing debate within the transplantation community as to which method is superior, but with the 1-year graft survival of heart-beating (HB) donor kidneys being comparable for the two methods of preservation, the less labor-intensive CS gained popularity [93, 101, 102]. Recently, however, there has been a resurgence of interest in the use of MP for kidney preservation because of its ability to reduce the occurrence of a delay in normal graft function, called delayed graft fuction (DGF), from 25% to 10% as compared to CS [23]. DFG calls for dialysis and longer hospitalization, and it appears to lead to a poorer long-term graft outcome [23, 36, 55, 81, 82].

It is very apparent that when one considers the graft outcomes that have been achieved with kidneys from young HB donors, cold preservation by either method is remarkably successful and has provided the foundation for the growth experienced in clinical transplantation. However, in recent years, the use of non-heart-beating (NHB) donor kidneys, where a prior insult of warm ischemia precedes the period of cold preservation, has been implemented in attempts to reduce the growing gap between the demand and supply of kidneys for transplantation [24, 29]. For these ischemically damaged kidneys, MP also seems preferable to CS [15]. In a clinical experiment, in which one kidney of a NHB donor was cold-stored and the other kidney machine-perfused, earlier graft function was observed in the latter [56, 67, 68]. Nevertheless, with the use of NHB donor kidneys, a dramatic increase in DGF and primary nonfunction (PNF) has been encountered as compared to HB donor kidneys [21, 30, 31, 40, 46, 53, 67, 75, 86, 107]. Cold preservation has, therefore, proven to be less effective when using NHB donors than with HB donors.

The need for prospective evaluation and improved preservation of NHB donor kidneys

It has been postulated that a more pharmacological approach to organ preservation might lead to an improvement in initial graft function [3]. MP offers the possibility of both pharmacological intervention and testing because the organ is continuously being perfused. Pefusion allows for the administration of agents, sampling of the perfusate, and monitoring of the perfusion dynamics during preservation.

Until now, pharmalogical agents have played a minimal role in clinical organ preservation, despite intensive research aimed at reducing ischemic damage. This is largely due to the fact that the pharmacological agents must already be present at the time of the ischemic insult and, therefore, are not relevant to a clinical situation. Southard [92] postulated that the agents are often inactive or insoluble at the hypothermic temperatures used in preservation, and that poor permeability inhibits adequate access to the sites where they are most needed. In addition, a combination of drugs may be needed to counteract the various stages of injuries at different sites since cell death is the consequence of a cascade of cellular injury.

Viability testing in clinical transplantation has gained renewed interest with the resurgence of the NHB donor. The extent of warm ischemic damage in these donors is often difficult to determine on the basis of clinical data alone. Historically, ex vivo evaluation of the viability status has focused on perfusion pressure and the corresponding perfusate flow for an interpretative vascular resistance. Differences in the values of glomerular filtration rate (GFR) and perfusate flow rate per time were used to distinguish prior warm ischemic-injured from non-injured kidneys. Lewis et al. [47] reported that deterioration in the ex vivo GFR and flow rate occurred sooner in warm ischemically damaged kidneys and later in nondamaged kidneys. This observation is not surprising when one considers that prior warm ischemic damage, compounded by cold ischemic exposure, would be expected to result in compromised function sooner than in kidneys with a single insult caused by metabolic inhibition from hypothermia.

In contrast to the attempts to use vascular perfusion characteristics as a marker of viability, Kootstra et al. have taken the inverse approach of evaluating the leakage of the intracellular enzyme alpha glutathione S-transferase (α-GST) into the extracellular space and, subsequently, the perfusate [51, 52]. The inhibition of metabolism caused by hypothermia results in the loss of cell surface polarity and normal cellular barrier function. Therefore, it seems logical that intracellular components leak into the extracellular spaces during organ preservation. The extent of alteration in permeability was found to correlate with the degree of prior warm ischemic exposure. However, α-GST has been reported to be confined to the proximal tubule. Tubular epithelium is known to have a remarkable capacity to regenerate following ischemic insult. While the α-GST marker is convenient for evaluating tubular damage, more informative viability markers that can differentiate between reversible and irreversible damage are needed for prospective organ evaluation.

Other approaches have been developed to aid in the determination of ischemic damage. These include energy charge, electrolyte concentrations, and functional studies. Yet, despite these efforts, there is currently no objective method or assay to evaluate the potential function of a renal allograft, making transplantation the only definitive test of graft viability [54]. An impediment to developing viability testing is the hypothermia during preservation. Hypothermia inhibits metabolism by over 95% and decreases oxygen consumption exponentially [12, 61], making evaluation of renal function virtually impossible. Organ preservation by means of hypothermia slows the ongoing extracorporeal ischemic/hypoxic damage rather than preventing or reversing such damage. Therefore, the result of the hypothermic-mediated inhibition of metabolism during preservation is the rate change in the processes leading to cell death [91]. Kootstra's group demonstrated that intermittent perfusion with normothermic blood in a canine transplant model extended the cold perservation times significantly, resulting in more viable kidneys at reimplantation as compared to cold preservation alone. It was postulated that the beneficial effect of this intermittent perfusion in the course of cold preservation could be attributed to a washout of metabolic waste products and a restoration of exhausted enzyme systems and energy sources [65, 105, 106].

The difficulties experienced with viability testing can also be attributed to the complex and multiple injury mechanisms prior to, and resulting from, the preservation period, as well as to the subsequent injury to which the kidney is exposed upon reperfusion. The deleterious effects of sudden restoration of oxygenation and blood flow to the cold, anoxic organ, leading to subsequent damage, makes it difficult to predict function prospectively.

The development of new and more physiological preservation technology could potentially lead to improved preservation and the development of viability assays for NHB donor kidneys. If such a theoretical technology could support adequate metabolism and corresponding function during the preservation period, then it might be feasible to develop viability testing that could distinguish between viable and nonviable organs. A more physiological preservation technology will need to focus on:

1. Facilitation of a polypharmacological approach during preservation to ameliorate ischemic injury;
2. Creating an environment in which the development of prospective testing during machine perfusion can be achieved;
3. Developing technology that can reverse ischemic damage during preservation rather than attempting to slow down the biochemical processes that lead to cell death;
4. Better understanding of the mechanisms involved in preservation-reperfusion injury.

Since hypothermia does not seem to provide the milieu for achieving these goals, the most promising and expedient principle appears to be organ preservation at warmer temperatures.

Kidney preservation at warmer temperatures

The idea of preserving an isolated portion of the body in order to examine its functions is not new. In 1812, physiologist Le Gallois wrote: "If one could substitute for the heart a kind of injection. ..of arterial blood, either natural or artificially made,. ..one would succeed easily in maintaining alive indefinitely any part of the body whatsoever" [58]. For more than 150 years, researchers have attempted to achieve this goal. In reviewing this body of work, it becomes apparent that previous attempts at mimicking a physiological preservation failed for two major reasons. First, failure to maintain the integrity and normal barrier functions of the vasculature leads to a rapid deterioration in vascular flow and a concurrent development of edema. Second, the inability to support adequate delivery of nutrients and oxygen leads to a deteriorating metabolic state. In light of these two major failures, the historical experience in preserving organs ex vivo at near physiological temperatures is described below.

Vascular integrity during warm temperature perfusion

In 1849, normothermic isolated kidney perfusion, using defibrinated blood, was initiated by Loebell [62]. In 1903, Brodie [20] designed a perfusion system for isolat-

ed organs by oxygenating the blood with air. Unfortunately, flow rates in the kidney were rarely sufficient. In 1914, Bainbridge and Evans managed to increase the renal blood flow and improve function during isolated perfusion by incorporating an animal lung into the circuit, postulating that the vasoconstrictive materials present or formed in the defibrinated blood were removed by the lung. Nevertheless, the survival of such a preparation was limited to approximately 6 h [4]. In 1925, active urine secretion was achieved during a 5-h period [94]. However, these early attempts to study the isolated kidney by means of a heart-lung kidney preparation had little success in keeping the organ alive, and its function remained depressed compared to physiological values.

Winton and co-workers (1940) extensively studied the kidney's physiology during isolated perfusion. Using a pump-lung-kidney preparation and an arterial pressure of approximately 100 mmHg, they examined inulin and creatinine clearance and the influence of the perfusion temperature [89]. Although the observed values were, for the most part, abnormally low, with kidneys only surviving for a few hours in good condition, they described a decrease in renal blood flow and creatinine clearance with cooling. Changes were found in the composition of urine that were comparable to that of serum transudate when reducing the temperature from 37 °C to 3 °–13 °C [12]. The observation of a decrease in renal blood flow with hypothermia was later confirmed by Levy, who attributed it to increased viscosity and vasoconstriction [61]. Kupfer et al. performed detailed studies of the pump-lung-kidney preparation, describing the impairment of glomerular filtration rate, para-aminohippuric acid clearance, creatinine urine/plasma ratio, and the rate of sodium excretion. The average survival of the preparation was 6 h [57].

Experiments by Nizet and Cuypers et al. revealed a severe deterioration in vascular circulation when blood was stored for 40 min, or if the blood was handled traumatically. They descibed a neutralizing effect of the vasoactive phenomenon by the lung, liver, and kidney itself [28, 74]. By reducing the mechanical damage to the blood, using newly procured blood, and by reducing the volume used, they claimed to have achieved near-normal kidney function in an ex vivo perfusion system [73].

The studies performed in the 1950s by Cough et al. [22, 27] focused on sustaining viability in excised kidneys at 25 °C for extended times without the use of a lung. They succeeded in maintaining urine production for up to 7 h by using blood-pump-oxygenator preparations. Subsequent attempts at reimplantation of these kidneys failed. Telander [99] successfully transplanted baboon and sheep kidneys following normothermic kidney perfusion with fresh heparinized blood for periods ranging from 5 to 7 h. The perfusate consisted of heparinized blood and a balanced salt solution (1:1) containing vitamins, the essential amino acids, and low molecular weight dextran. Post-transplant function, evaluated after contralateral nephrectomy performed within 21 days of the reimplantation, proved to be relatively normal. They additionally perfused kidneys for at least 24 h without reimplantation, suggesting the feasibility of prolonged warm perfusion by diluting the perfusate with a balanced salt solution and adding low molecular weight dextran. Thus, although some obstacles were overcome, physiological studies using the isolated kidney perfusions with blood had their limitations, including brevity of the steady-state period, low glomerular filtration rates, abnormal tubular functions, alterations in intrarenal blood flow distribution, and limited duration of survival [39, 72].

In the 1960s, several investigators established the correlation between platelets, blood cell aggregates, and degradation products during blood perfusion and reported the difficulties experienced with rising perfusion pressure, tissue edema, and impaired function or no function upon reimplantation of the organ [8, 16, 63, 96]. To avoid these difficulties, plasma was selected as a perfusate. The first attempts involved using plasma diluted in a 1:3 ratio with an electrolyte solution. The diluted plasma resulted in minimal edema and a minimal increase in perfusion pressure. However, the organs proved to be nonviable after transplantation. When undiluted plasma was used, a recurrence of the rising perfusion pressure, severe edema, and tissue destruction was seen. When frozen sections of the kidney were taken during perfusion, multiple small emboli were seen in the arterioles, in addition to fat droplets in the tubules and intratubular cells [7]. The rising perfusion pressure was attributed to obstruction of the vessels by lipid components released into the perfusate by denaturation. The occurrence of fat emboli during warm perfusion appeared to be less when a membrane oxygenator was used in place of a film oxygenator. It was postulated that the low-density lipoproteins aggregated into larger particles due to damage. Belzer's group found a beneficial effect of denaturation of the lipoproteins by freezing and quick-thawing prior to usage (cryoprecipitated plasma). This process removed about 30 %–35 % of the lipid components, believed to be mainly low-density lipoproteins that exhibited less stability than high-density lipoproteins. No fat emboli could be found after this filtration [7].

Oxidative metabolism during warm preservation

In subsequent years, several investigators attempted to preserve kidneys at 25 °C by means of a perfusion apparatus and an acellular perfusate modeled after that described by Belzer et al. This perfusate consisted of the homologous pooled plasma, cryoprecipitated and filtered, to which was added hydrocortisone, sodium peni-

cillin, insulin, magnesium sulfate, dextrose-water solution, and mannitol. The additives in the perfusate were chosen on an empirical basis. Magnesium and insulin were added to mimic hibernation, as described by Suomalainen [95]. Penicillin was used against possible infections, and the steroids as membrane stabilizers [64]. The dextrose and mannitol were used to maintain osmotic pressure. The arterial pressure was maintained at 60–95 mmHg, with the pH in the range 7.3–7.5, and the pO_2 above 400 mmHg. Clearance values were relatively low: approximately 20% of the normal values for in vivo kidneys [10, 11, 87]. All researchers described a decrease in tubular function occurring between 2 and 4 h of preservation when using an acellular perfusate. Supporting these finding were Malinin et al.'s observations [66] showing a 57% reduction of intact glomeruli after 4 h perfusion at 25 °C, compared to a 21% reduction in organs perfused at 5 °C. At 25 °C the ongoing renal oxidative metabolism during preservation varies from 50% to 75% of the physiological oxygen consumption. These findings suggest that the oxygen required by the organ during preservation at elevated temperatures cannot adequately be provided by the oxygen dissolved in an acellular perfusate [61], thus leading to anoxic damage after 2–4 h of perfusion. Since warm perfusion appeared feasible only for short periods of time, it was mainly used experimentally as an intermittent warm perfusion, attempting viability testing of the organ, during hypothermic preservation.

In terms of whether warm temperature perfusion could provide the basis for developing viability testing, the work of Pegg et al. [38] suggested that renal metabolism and function during warm perfusion was sufficient to enable comparisons between test groups in terms of tubular secretion and reabsorption. Following 24 h of hypothermic preservation, they attempted to estimate renal function by in vitro normothermic perfusion. Using a bloodless perfusate, kidneys were perfused at 37 °C for 2 h. All kidneys demonstrated impaired renal function during perfusion, such as increased permeability resulting in proteinuria. None of the kidneys were reimplanted and outcomes were not determined. In spite of this, differences in function could be determined, and these results support the concept that viability testing could be developed if the perfusion temperature was sufficient to support ongoing cellular metabolism.

A sufficient oxygen supply with an acellular perfusate can be achieved by adding an oxygen carrier, such as a hemoglobin preparation or a perfluorochemical, to the preservation solution. Early trials with solutions containing hemoglobin proved to be unsuccessful, mainly due to the development of kidney damage similar to pigment nephropathy seen after massive hemolysis or rhabdomyolysis [1, 2]. With the development of highly purified stroma-free hemoglobin (SFH), less toxic side effects were observed, leading to the assumption that the contamination with erythrocyte stroma was responsible for the previously observed toxicity [13, 37, 76–79, 103]. Nevertheless, several investigators described a continuous decrease in flow when perfusing kidneys with a SFH solution, with histology revealing hemoglobin deposits in the glomeruli [84] and tubules [9]. Other investigators confirmed these adverse effects on kidney function using ultrapure SFH, which was not observed with polymerized hemoglobins [44, 59, 83, 98]. Confirmation of the low-grade toxicity to the kidney using different purified and polymerized or cross-linked hemoglobin derivatives was obtained in several studies. Minimal reductions in the clearance and reabsorptive functions of the kidney were observed [34, 35, 97] along with moderate structural alterations [34, 60, 69, 90, 97], all of which were reversible. This led to the conclusion that the hemoglobin molecule itself had a toxic effect by passing through the glomerular filter. Support for this interpretation was provided by the findings of Tam and Wong [98], showing that polymerization of hemoglobin into very large macromolecules prevented passage through the glomerular filter. In a study performed by Willinger et al. [108], ultrapure polymerized bovine hemoglobin was used to evaluate the structural and functional integrity of isolated perfused rat kidneys. They demonstrated a beneficial effect of the polymerized hemoglobin on kidney function, morphology, and oxygenation in comparison to control kidneys perfused with a hydroxylethyl starch solution. Therefore, polymerized hemoglobin appeared to be a promising oxyphoretic additive for the perfusion of rat kidneys.

Horiuchi et al. described 12-h normothermic canine kidney perfusion with a pyridoxalated hemoglobin-polyoxyethylene (PHP) solution in combination with UW solution [45]. PHP is a conjugate of human hemoglobin with alpha-carboxymethyl and omega-carboxymethoxyethylene, used because of its longer half-life time and higher oxygen-transporting capacity [48]. Although PHP provided a stable flow and pressure, histology revealed 30% to 40% tubular damage. None of the kidneys were reimplanted.

Advances in perfluorochemical (PFC) emulsion technology provided another class of oxygen carriers. PFCs differ from hemoglobin preparations in that they are totally synthetic compounds on a liquid hydrocarbon base. In contrast to hemoglobin, oxygen is not chemically bound to the PFC carrier. PFCs take up and release oxygen following Henry's linear law, based on the partial pressure of the gas, rather than Barcroft's sigmoid curve described for hemoglobin [80]. Unlike hemoglobin, acidosis, alkalosis, 2,3-diphosphoglycerate, and temperature seem to have no or little effect on the oxygen delivery of PFCs. PFC molecules do not mix with water, and it is therefore necessary to emulsify them for intravascular usage. Early emulsions were quite ineffective, but problems with the stability of the emulsion were solved

by improving the adhesion of the surfactant film to the fluorocarbon droplet [104]. These second-generation PFC emulsions possessed a much greater PFC concentration, a lower toxicity profile, and the potential for providing physiological concentrations of oxygen.

Early studies involving the perfusion of kidneys with PFC were reported by Beisang et al. in 1970 [5]. In 1975, Nakaya et al. perfused rabbit kidneys at room temperature for 9 h with a PFC emulsion [71]. Overall, oxygen delivery and renal metabolism were better maintained with PFC supplementation than with a perfusate consisting of electrolytes alone. However, none of these kidneys were reimplanted. Other studies during the 1970s confirmed the possibility of perfusion with a PFC solution, although histological abnormalities were seen after approximately 5 h of perfusion [43]. In the 1980s Dunn and colleagues managed to perfuse kidneys with a PFC emulsion for 18 h, but the development of edema and a corresponding increase in vascular resistance led to the loss of organ viability [32]. Kawamura et al. [49] managed to perfuse damaged and undamaged rabbit kidneys at 25 °C with a perfusate formulation similar to human extracellular fluid supplemented with a PFC (3% v/v). A perfusion pressure of 80 mmHg proved to be most suitable for studying the differences in physiological dynamics between the ischemically damaged and undamaged kidneys. No kidneys were transplanted after the 12 h of perfusion.

Recently, successful ex vivo canine kidney preservation at temperatures of 25 °–32 °C for 6–18 h has been described by Brasile [17]. This warm preservation method is based on a perfusate developed from a modified tissue culture medium and a PFC emulsion (perflubron) as an oxygen carrier. In the experiments described by Brasile et al., the canine kidneys perfused at 25 °C and 32 °C, respectively, demonstrated stable flow dynamics, diuresis, glucose, and oxygen consumption. The urine produced was free of protein and glucose, and histological evaluation of the kidney showed intact glomeruli and tubuli with no evidence of edema. Six kidneys were successfully transplanted following 6 h of perfusion at 25 °C. This warm perfusion is proposed to enable functional evaluation of kidneys prior to transplantation, to resuscitate kidneys following warm ischemic damage equivalent to NHB donor kidneys, and to prevent reperfusion injury [18, 19, 41, 42].

Preservation into the next millenium

It is logical to consider that an in vitro measurement during warm temperature perfusion that mirrors the physiological functions of kidneys in vivo would provide the best means of distinguishing between viable and nonviable organs prospectively. This would most likely require multiple assays that form a score that predicts PNF better than one analysis alone. The viability testing could be coupled with the traditional cold preservation. A cold-preserved organ could be transitioned to warm perfusion and the restored metabolism used to evaluate function prior to transplantation.

There are two areas in organ transplantation where advancements in preservation-reperfusion phenomenon will be important. The first is the elimination of the damaging effects of cold preservation. The second is the amelioration of injurious events during reperfusion. The manifestation of the injury sustained during warm ischemia and hypothermic preservation are now only observed upon reperfusion of the organ. Reperfusion injury is, in part, the direct result of the increase in temperature and re-exposure to a high-oxygen tension following hypothermic preservation. The development of near-normothermic preservation technology may make it possible to greatly reduce this reperfusion injury. Additionally, warm temperature preservation could provide a mechanism to repair warm ischemic damage by utilizing pharmacological agents to enhance cellular metabolism.

Obviously, many obstacles will need to be overcome before warm perfusion preservation can become a reality. Issues pertaining to potential contamination, how warm perfusion would interface with traditional hypothermic preservation, mechanisms to support metabolism, and what would be needed to maintain the organs under such conditions for extended periods of time are just a few of the areas that will need to be addressed. Assuming these substantial obstacles can be overcome, there is evidence from several groups of researchers that warm temperature organ preservation would be beneficial in providing a reproducible viability assay and, perhaps, in diminishing DGF in NHB donor kidneys.

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