Adenosine treatment augments random flap survival in rats

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Adenosine and purine nucleosides are intermediates in the pathway of purine nucleotide degradation. Adenosine causes vasodilation in all arterioles, except those in the kidney, and is the major regulator of coronary blood flow. The objective of the present study was to investigate the efficacy and role of 9-beta-D-ribofuranosyl adenosine (RFA), a derivative of adenosine, for the augmentation of random flap survival in rats. Varying doses of adenosine and a nonselective adenosine antagonist, 8-phenyltheophylline, were administered before elevation of 3×10 cm dorsal random flaps in 60 rats. The rats were randomly assigned to five groups and received the following treatment: group I (controls) was treated with placebo (saline, 1 mL/day); group II was treated with RFA 25 μg/kg/day; group III was treated with RFA 50 μg/kg/day; group IV was treated with RFA 100 μg/kg/day; and group V was treated with 8-phenyltheophylline (10 mg/kg/day). All daily injections were given intravenously for seven days. Flap survival was assessed on day 8. Therapeutic and higher doses of adenosine-treated flaps showed a significant increase in viability compared with saline-treated flaps in the control group, while there was no improvement in flap survival with low dose adenosine. Phenyltheophylline reversed the beneficial effect of adenosine and increased flap necrosis, which was comparable with that of the controls. The findings show that adenosine can enhance flap survival, and this beneficial effect is possibly due to vasodilation, inhibition of noradrenalin release, reduction of energy consumption, inhibition of reactive oxygen species and a preconditioning effect; however, this effect seems to be dose-related. Adenosine is an easily available drug for clinical use in ischemic heart diseases and should be considered in potentially ischemic flaps.

Key Words: *Adenosine; Flap survival; Theophylline*

Le traitement à l'adénosine prolonge la survie de lambeaux aléatoires chez le rat

RÉSUMÉ : Les nucléosides de l'adénosine et de la purine sont des intermédiaires dans la voie de dégradation nucléosidique de la purine. L'adénosine provoque une vasodilatation de toutes les artérioles à l'exception de celles du rein, mais agit à titre d'important régulateur du débit cardiaque coronarien. L'objectif de la présente étude était de mesurer l'efficacité et le rôle de la 9-bêta-D-ribofuranosyl adénosine (RFA), un dérivé de l'adénosine, pour prolonger la survie d'un lambeau aléatoire chez le rat. Des doses diverses d'adénosine et de l'antagoniste non sélectif de l'adénosine, 8-phénylthéophylline, ont été administrées avant le soulèvement de lambeaux aléatoires dorsaux de 3 cm sur 10 chez 60 rats. Les rats ont été assignés aléatoirement à cinq groupes et ont respectivement reçu les traitements suivants : groupe I (témoins) placebo (solution physiologique, 1 mL/jour); groupe II, RFA 25 μg/kg/jour; groupe III RFA 50 μg/kg/jour; groupe IV, RFA 100 μg/kg/jour et groupe V, 8-phénylthéophylline (10 mg/kg/jour). Toutes les injections quotidiennes ont été administrées par voie intraveineuse pendant sept jours. La survie des lambeaux a été évaluée au jour 8. Les lambeaux traités avec les doses thérapeutiques et supérieures d'adénosine ont connu une augmentation significative de leur viabilité comparativement aux lambeaux traités par solution saline dans le groupe témoin, bien que l'on n'ait noté aucune amélioration de la survie des lambeaux avec les doses plus faibles d'adénosine. La phénylthéophylline a renversé les effets bénéfiques de l'adénosine et augmenté la nécrose des lambeaux qui s'est révélée comparable à celle des témoins. Les conclusions montrent que l'adénosine peut améliorer la survie des lambeaux; cet effet favorable est probablement dû à la vasodilatation, à l'inhibition de la libération de noradrénaline, à la réduction de la consommation d'énergie, à l'inhibition des espèces réagissant à l'oxygène et à un effet préconditionnant. Par contre, cet effet semble lié à la dose. L'adénosine est un médicament facilement accessible pour usage clinique dans les maladies cardiaques ischémiques et elle est à envisager dans les cas de lambeaux potentiellement ischémiques.

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While skin flaps are frequently used in surgical practice, the pathophysiology of their occasional failure is poorly understood. An increasing number of studies is devoted to the pharmacological manipulation of flaps, yet the exact physiological basis for the use of these agents is unsolved (1). Tremendous research efforts have been directed toward identifying pharmacological interventions for the augmentation of skin flap survival, and every surgeon would welcome an agent that minimizes the deleterious effects of ischemia or enhances the tolerance of the tissue under ischemic conditions. A recent trend in flap research is aimed at increasing the tolerance of the tissue to warm ischemia and attenuation of the necrosis (2-3).

Adenosine, which is derived from the metabolism of adenosine triphosphate (ATP), is a potent vasodilator that acts on specific receptors located on the outer surface of the smooth muscle cell (4-5). The nucleotides ATP, adenosine 5′-diphosphate and adenosine monophosphate, and the nucleoside adenosine have been shown to induce coronary vasodilation and accumulate rapidly in large amounts in the ischemic myocardium (5). Adenosine is a very inexpensive and safe clinical drug, and it can be administered conveniently without any serious systemic effects (6). Several studies have reported that adenosine treatment attenuates infarct size in muscle flaps and displays the major role as the 'trigger mediator' of acute ischemic preconditioning in skeletal muscle and in the heart (7-8).

Therefore, the objective of the present project was to investigate the efficacy of acute adenosine treatment for the attenuation of ischemic necrosis in random skin flaps in the rat.

MATERIALS AND METHODS

Sixty Wistar albino rats, weighing 275 to 375 g, were used in the present study. The rats were anesthetized with intraperitoneal injections of ketamine (50 mg/kg) and xylazine (5 mg/kg). Borders of the random flaps were outlined on the back of the animals by using a flap template. Caudally based 3×10 cm dorsal skin flaps were raised in rats, according to Khouri et al (9) and resutured to the original site with 4-0 silk sutures. No electrocautery or hemostatic agents were used. Each rat was housed in a separate cage at 22°C to prevent cannibalism, and had free access to water and food. The following agents were used: 9-beta-D-ribofuranosyl adenosine and 8-phenyltheophylline.

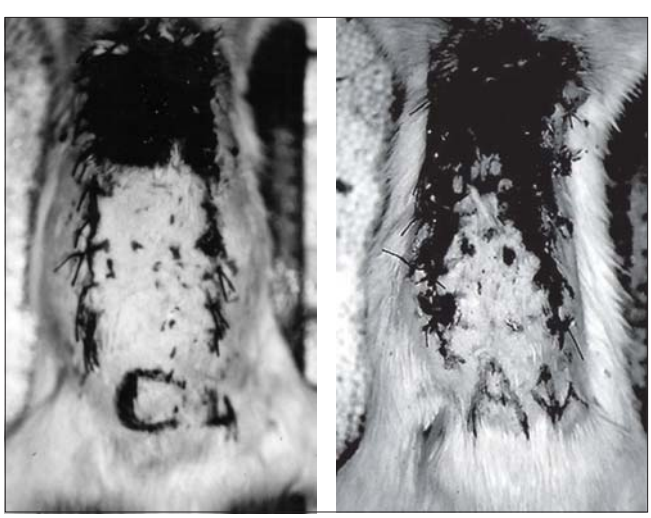

Figure 1) Left *Photograph of a representative flap illustrating the regions of survival and necrosis from the control group of rats treated with saline.* **Right** *Increased necrosis noted in a representative flap treated with low dose adenosine*

The rats were randomly assigned to five groups. Group I (n=12), the control group, was given saline 1 mL/day intravenously. Group II (n=12) received adenosine 25 μg/kg/day intravenously. Group III (n=12) received adenosine 50 μg/kg/day intravenously. Group IV (n=12) received adenosine 100 μg/kg/day intravenously. Group V (n=12) received 8-phenyltheophylline 10 mg/kg/day intravenously.

The first dose of all the drugs used in the present study was administered 10 min before elevation of the flaps. Three different doses of adenosine (25, 50 and 100 μg/kg) were chosen according to previous articles that reported a reduction in the infarct size by the aforementioned doses (7,8,10,11). Injection of saline or adenosine was continued for one week postoperatively. No other medication or antibiotics were given.

Evaluation

Flap survival was assessed at the end of the first postoperative week in all animals. While the rats were under anesthesia, the flaps were traced on acetate paper, and the area of necrosis was marked. The area of necrosis and total flap area were measured using a polar planimeter. The surface area of flap necrosis, represented by the blackened and indurated area of the cephalad part of the flap, was calculated using the planimeter, and the result for each flap was expressed as percentage survival (ie, surviving flap area/total flap area ×100). Values are expressed as means ± SEM. The student's *t* test was applied to the statistical analysis of independent samples, comparing the control group with each of the treated groups. Probabilities of less than 0.01 were accepted as significant. Minitab statistical software (Minitab Inc, USA) was used for statistical calculations.

RESULTS

None of the flaps was lost due to hematoma, infection or cannibalism. Control group rats treated with saline (placebo) had a percentage mean survival area of 69.92 (SEM=0.45) (Figure 1). Positive controls receiving theophylline (group V), which selectively antagonizes the beneficial effects of adenosine, had a significantly lower flap survival area ($P<0.05$), with a percentage mean survival area of 70.42 (SEM=0.85) (Figure 1). Rats receiving standard (group III) and high doses of adenosine (group IV) showed significantly increased flap survival compared with theophylline-treated flaps ($P<0.01$)

Low dose adenosine did not enhance the viability of random flaps, and flap survival was significantly less than that seen with standard and high dose adenosine-treated flaps, with a percentage mean survival area of 70.92 (SEM=0.57)

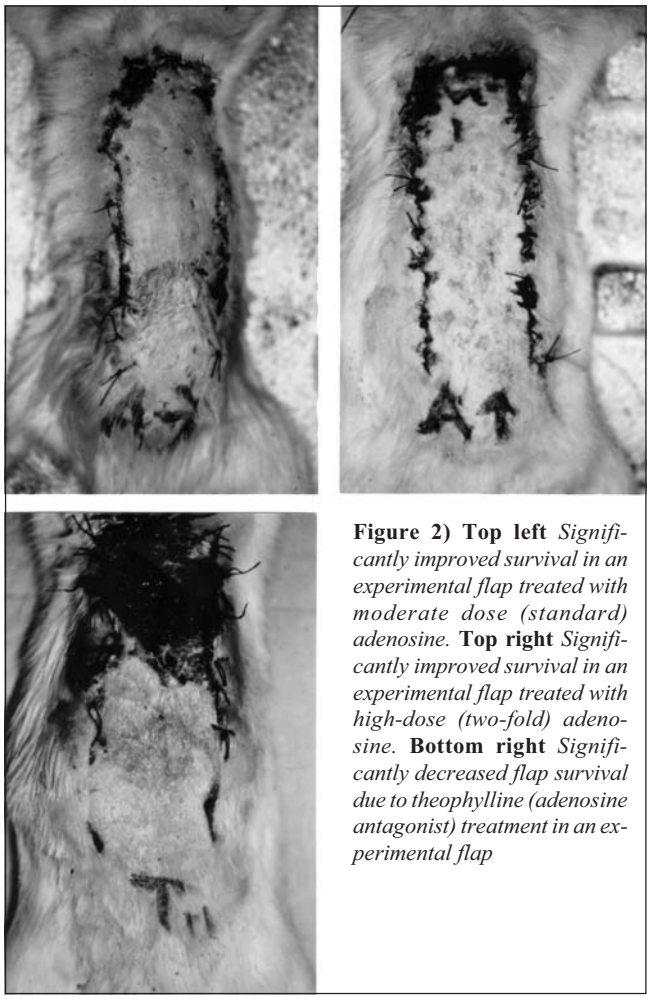

Figure 2) Top left *Significantly improved survival in an experimental flap treated with moderate dose (standard) adenosine.* **Top right** *Significantly improved survival in an experimental flap treated with high-dose (two-fold) adenosine.* **Bottom right** *Significantly decreased flap survival due to theophylline (adenosine antagonist) treatment in an experimental flap*

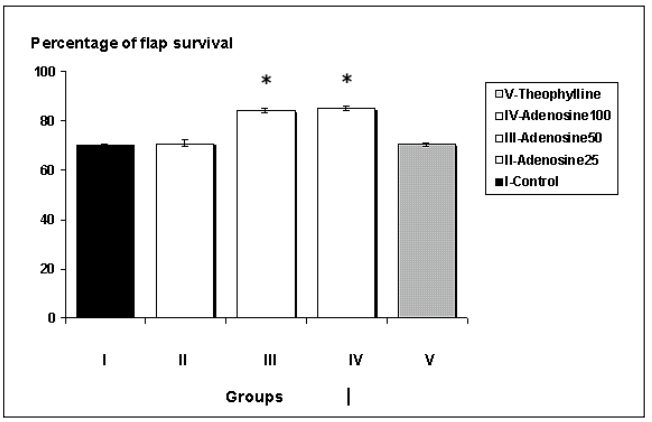

Figure 3) *A comparison of the values for mean per cent flap survival among the various doses of adenosine (25, 50 and 100 μg/kg/day), theophylline (10 mg/kg/day) and the control group (1 mL/day). *P<0.01 Percentage survival of flaps*

(Figure 2). Comparison of low dose adenosine-treated flaps with the flaps of controls yielded an insignificant difference in survival (P=0.18). Standard (group III) and high dose adenosine-treated (group IV) flaps showed a significantly augmented flap survival with mean survival areas of 84.0 (SEM=0.72) and 85.0 (SEM=0.61), respectively (Figure 2). There was no significant difference between groups III and IV in mean survival rate (P=0.383). Surviving percentage areas of the flaps in groups I to V are listed in Figure 3.

DISCUSSION

The present research has generated several observations. Specifically, therapeutic (standard) and two-fold high doses of intravenous adenosine administration were effective in augmenting flap survival in random-pattern flaps in the rat. It was also observed that this salutary effect of adenosine against necrosis in random dorsal flaps was reversed by an adenosine antagonist, 8-phenyltheophylline, that caused a necrosis pattern similar to that of untreated controls. Furthermore, the beneficial effect of adenosine seems to be dose-related because no enhanced flap survival was shown with low dose adenosine (Figures 2,3). The caudally-based, 3×10 cm sized, random flap in the rat was selected as the experimental model because its consistency and standardization were validated by Khouri et al (9). Area and pattern of flap necrosis in the control animals receiving saline were in conformation with previous studies.

A large body of evidence suggests that adenosine can protect the heart against ischemia and/or reperfusion injury (11,12). Many studies have shown that the activation of adenosine A_1-receptors showed an ischemic 'preconditioning' effect to reduce infarct size, and the blockade of these receptors abolished this effect (13,14). Pretreatment with adenosine has been reported to stimulate glycolysis in hypoxic hearts and delay the onset of ischemic contracture (15,16). The protective effect of adenosine in myocardial ischemia is achieved by regulating the myocardial oxygen supply-demand balance. During the period of oxygen deprivation, adenosine augments energy production through increased glycolytic flux and, during reperfusion, revives cellular energy charge via purine salvage pathways (17,18). Adenosine displays a receptor-mediated cardioprotective effect, and the activation of A_1-receptors couples a variety of effector systems in the myocardium. Activated A_1-receptors can couple to adenylate cyclase through an inhibitory G-protein, resulting in the inhibition of the conversion of ATP to cyclic adenosine monophosphate, resulting in preservation of ATP stores (18). These activated A_1-receptors can also couple to ATP-sensitive potassium ion (K_{ATP}) channels, probably by enhancing the channel sensitivity to ATP. Opening of K_{ATP} channels results in the increase of potassium ion influx and membrane hyperpolarization, thereby reducing the opening time for voltage-dependent calcium channels and, thus, resulting in the shortening of action potentials and slowing of cellular ATP catabolism, and increasing myocardial ischemic tolerance (19,20). Forrest and colleagues (7) showed that preischemic adenosine treatment is effective in the augmentation of ischemic tolerance in muscle flaps, and this protective effect was attributed to the activation of A_1-receptors and K_{ATP} channels, resulting in diminished metabolism and slowed ATP consumption.

Besides its direct effect on myocytes, adenosine has been

shown to attenuate noradrenalin release from sympathetic nerves during early ischemia and antagonize the positive inotropic effect of catecholamines mediated via the adenylyl cyclase system (10). These effects would compromise the oxygen demand of the flap during ischemia and decrease the rate of ATP degradation. The inhibition of noradrenalin may be another mechanism through which adenosine has augmented random flap survival in the rat because sympathetic regulation of the circulation within the flap was shown to have a significant role in the maintenance of flap survival (21,22). Furthermore, adenosine A_2-receptors stimulate adenylate cyclase through a stimulatory G-protein, causing myocardial vasodilation (17,23). Vasodilation that resulted from adenosine A_2-receptor stimulation may also be beneficial in increasing critical blood supply to the flap before and after ischemia, thus increasing the surviving area.

Adenosine has also been shown to inhibit superoxide anion production from neutrophils, and inhibit platelet aggregation and microthrombi formation in the capillaries (24,25). Beneficial effects of prevention or clearance of free oxygen radicals in states of ischemia have been shown in several studies, and enhanced flap survival in the present study can be attributed to this effect of adenosine, whereas inhibition of platelet aggregation and antithrombotic treatment have been shown to decrease distal flap necrosis in numerous studies (26,27). Minamino et al (28) have shown that adenosine treatment inhibits leukocyte-induced vasoconstriction by inhibiting the adhesion of neutrophils to the endothelium via the stimulation of A_{2a}-receptors. This effect can be linked to the inhibition of lipid peroxidation by adenosine to contribute to the flap survival, and finally, adenosine receptor activation has been shown to stimulate the induction of vascular endothelial growth factor – a factor that has been shown to enhance flap survival through neovascularization. Though the 'graft effect' of the bed at the flap-bed interface is controversial, many studies that have used systemic or topical factors that trigger neovascularization have been proved to be salutary on random flap survival, so, apart from its direct vascular effects, adenosine can be hypothesized to enhance the revascularization of the severely-ischemic distal random segment of the flap (29,30).

In the present experiment, adenosine, which is also used clinically as a coronary vasodilator, was hypothesized to show a beneficial effect by decreasing distal flap necrosis because adenosine was previously shown to increase tolerance to ischemia in the myocardium and skeletal muscle. Flap survival was clearly enhanced by adenosine in varying doses, and to double-check the hypothesis, a selective adenosine antagonist, phenyltheophylline, which was shown to reverse and abolish the preconditioning and cardioprotective effects of adenosine in myocardium, was also administered (31). 8-phenyltheophylline strongly abolishes the vasodilatory effect of adenosine and adenosine 5′-diphosphate through P2Y1 receptors and decreases total coronary resistance in rats (5).

The mechanisms underlying the success of adenosine in reducing flap necrosis was not the main goal of the present study and, therefore, these mechansims were not investigated. To the authors' knowledge, the present study is the first to investigate the use of adenosine in a field other than myocardial ischemia or muscle flaps. It is most likely that adenosine exerts its salutary effect through the combined inhibition of ATP catabolism, noradrenalin release, inhibition of lipid peroxidation, increased release of vascular endothelial growth factor and, most importantly, vasodilation. Further studies are required to delineate the exact basis for this salutary effect on random flap survival in rats. These findings support another clinical use of adenosine for flap salvage apart from its use in the salvation of ischemic heart disease.

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