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REVIEW

The Role of Pharmacokinetics and Pharmacodynamics in Early Drug Development with reference to the Cyclin-dependent Kinase (Cdk) Inhibitor - Roscovitine

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دور حركية وديناميكية الأدوية في الجسم في التطوير المبكر للعلاج قياساً على مثبطات السايكلين المعتمد على الكاينيز (روسكوفيتين)

مصطفى حسن، حاتم سلام، سوزانا حسن

الملخص: تلعب دراسة حركة وديناميكية الأدوية وعلم الوراثة الدوائي دوراً مهما في اكتشاف العقاقير الطبية وتساهم كذلك مساهمة فعالة في نجاح العلاج، وهذا ما يجعلها ضرورية عند استخدام العقاقير في علاج الأمراض السرطانية نظراً لسميتها العالية. خلال العقد الماضي تم التعرف على مثبطات السايكلين المعتمد على الكاينيز (سي دي كي) كمركبات فعالة في علاج الكثير من الأمراض، بالإضافة إلى بعض الأمراض السرطانية. ومن وظائف مركبات ال (سي دي كي) الأساسية في الجسم دورها في تنظيم دورة انقسام الخلية واستنساخ الحامض النووي الرايبوزي. وقد وجدت علاقة سببية بين الخلل في تنظيم عمل تلك المركبات وزيادة نسبة حدوث بعض الأورام الخبيثة، وبعض الأمراض التنكسية العصبية بالإضافة إلى زيادة القابلية للعدوى الفيروسية أو الطفيلية، وزيادة معدلات الإصابة بمرض التهاب كبيبات الكلى وغيرها من الالتهابات المرضية. (آر– روسكوفيتين) هو مركب مستحدث معمليا باستبدال ثلاثة أماكن من حلقة البيورين بثلاثة مجموعات أخرى. وقد ثبت أن تأثيره موجه ضد ال (سي دي كى 1، 2، 5، 7 و 9) دون غيرها. ومن خلال الأبحاث المعملية أظهر الروسكوفيتين مقدرة واعدة في القضاء على الخلايا والأورام السرطانية المستزرعة. نقدم في هذه الورقة البحثية عدة مفاهيم عن حركية وديناميكية الروسكوفيتين في الجسم. كذلك أوضحنا بعض نتائج دراستنا المتضمنة التحليل الحيوي، تسمم الدم، تأثير العمر على حركية الدواء، وحركية وتأثير مركبات ال (سي دي كى) في الدماغ. وقد استنتجنا أن عدم تجانس نتائج معايير حركية الدواء وسوء توزيعه في مكونات نخاع العظم ربما يوضح عدم فاعلية تثبيط الدواء لخلايا النخاع العظمي داخل الجسم بالرغم من فعاليته في الفحص المخبري.النتائج المؤكدة على تعرض المصل والدماغ لنسبة عالية من الدواء مع طول فترة التخلص منه في الجرذان الوليدة مقارنة بالجرذان البالغة تعزز من احتمالية استخدام الروسكوفيتين لعلاج أورام الدماغ عند الأطفال في المستقبل. تثبيط ال (سي دي كى 5) وتنشيط (ايرك 1 و 2) المكتشفة في دماغ الجرذان الوليدة يرجح استعمال الروسكوفيتين في علاج الأمراض التنكسية العصبية. الدراسات المبكرة لحركية وديناميكية الأدوية هي من الأمور المهمة لاكتشاف عقاقير جديدة واعدة.

مفتاح الكلمات: حركية الأدوية، ديناميكية الأدوية، روسكوفيتين، مثبط ال (سي يدي كي)، عقاقير ضد السرطان، تسمم، الحركية المعتمدة على العمر.

ABSTRACT: Pharmacokinetics, pharmacodynamics and pharmacogenetics play an important role in drug discovery and contribute to treatment success. This is an essential issue in cancer treatment due to its high toxicity. During the last decade, cyclin-dependent kinase inhibitors were recognised as a new class of compounds that was introduced for the treatment of several diseases including cancer. Cyclin-dependent kinases (Cdks) play a key role in the regulation of cell cycle progression and ribonucleic acid transcription. Deregulation of Cdks has been associated with several malignancies, neurodegenerative disorders, viral and protozoa infections, glomerulonephritis and inflammatory diseases. (R)-roscovitine is a synthetic tri-substituted purine that inhibits selectively Cdk1, 2, 5, 7 and 9. Roscovitine has shown promising cytotoxicity in cell lines and tumor xenografts. In this paper, we present several aspects of pharmacokinetics (PK) and pharmacodynamics (PD) of roscovitine. We present also some of our investigations including bioanalysis, haematotoxicity, age dependent kinetics, PK and effects on Cdks in the brain. Unfavourable kinetic parameters in combination with poor distribution to the bone marrow compartment could explain the absence of myelosuppression *in vivo* despite the efficacy *in vitro*.Higher plasma and brain exposure and longer elimination half-life found in rat pups compared to adult rats may indicate that roscovitine can be a potential candidate for the treatment of brain tumours in children. Cdk5 inhibition and Erk1/2 activation that was detected in brain of rat pups may suggest the use of roscovitine in neurodegenerative

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diseases. Early pharmacokinetic/pharmacodynamic studies are important issues in drug discovery and may affect further development of promising drug candidates.

Key words: Pharmacokinetics; Pharmacodynamics; Roscovitine; Cdk inhibitor; Anticancer drugs; Toxicity; age-dependent kinetics

Clinical approval of new drugs is preceded by intensive research consisting of two steps, drug discovery and drug development. In the drug discovery stage, target enzymes and/or receptors for a particular disease are identified, and new molecules are designed and screened for their biological activities. Promising drug candidates are evaluated for their toxicity and efficacy in the development stage.[1]

Studies on the metabolism and pharmacokinetics (DMPK) of candidate drugs have become an essential part in drug discovery and development programmes and start usually concomitantly with the screening for biological activity. It is estimated that approximately 10–40 % of drug candidates fail due improper pharmacokinetic properties. Further, DMPK studies provide vital information about the PK/PD (pharmacokinetics/pharmacodynamics) relationship.[2] This knowledge is prerequisite for safety phase I clinical trials[3] and prediction of a clinical effective dose.

Moreover, PK is essential for further investigation of drugs in phase II and III clinical trials. PK/PD also play important roles in dose optimisation, personalised treatment and prevention of side effects. This in turn determines the clinical outcome. These aspects are especially important in anticancer drugs due to their toxicities and the narrow therapeutic window.

Age is one of the important factors affecting the PK and efficacy of drugs. Variability in drug exposure and efficacy occurs among the different age populations, i.e. children, adults and elderly. It is essential to investigate the PK parameters in the age population that is treated with the drug. In the younger aged population, doses of many drugs are still not optimised due to lack of knowledge about drug disposition and PK in this particular population. Several factors such as the ontogeny of the metabolising enzymes and drug transporters are responsible for the variability of DMPK.[4] Continuous efforts are being made to develop accurate PK models to predict the PK parameters in this population without conducting a large scale investigation which might be difficult due to technical and ethical restraints.[5] Age-dependent pharmacokinetics in young animals at different stages of development should be considered before clinical use.[6]

Cyclin-Dependent Kinases

Cyclin-dependent kinases (Cdks) are a family of serine/threonine kinases that are activated through binding to regulatory subunits called cyclins.[7] Cdk enzymes are homologues and highly conserved in their cyclin binding domain. Despite the fact that the human sequencing programme has successfully indentified 20 Cdks and 25 cyclins, their functions are still not fully understood and a limited number of active Cdk/cyclin complexes have been identified so far.[7]

Cyclin-dependent kinases are regulated by several mechanisms[7] including, transcription and translation of their subunits, heterodimerisation with cyclins, post-translational modification by phosphorylation and dephosphorylation and interactions with the natural inhibitors. The natural inhibitors CIP/KIP (p21, p27, p57) suppress the Cdk/cyclin complexes and INK4 proteins (p15, p16, p18 and p19) inhibit the Cdk4 and Cdk6 monomers.

Cdk/cyclin complexes play an essential role in the regulation of the cell cycle progression. Cyclins transcription and degradation varies during the different phases of the cell cycle and lead to the activation or inactivation of the corresponding Cdks.

During the last decade, the roles of Cdks in several diseases including cancer were extensively studied. Over expression of cyclin B1 and hyperactivation of Cdk1 has been observed in a number of primary tumours including breast-, colon- and prostate carcinoma. Inactivation of Cip–Kip inhibitors and over expression of cyclin E and/or cyclin A lead to deregulation of Cdk2 in various malignancies, including melanoma, ovarian

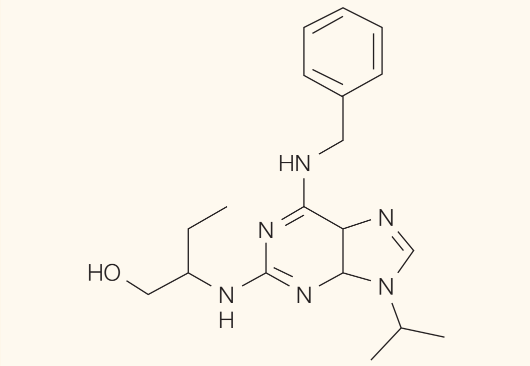

Figure 1: The structure of roscovitine and other members of the 2, 6, 9 trisubstituted cyclin-dependent kinase inhibitors (Cdki)

carcinoma, lung carcinoma and osteosarcoma.[8] Cdk5 has been found to modulate the metastatic potential of some malignancies including breast and prostate carcinomas.[9,10]

Cdk2 and Cdk5 have been shown to play important roles in apoptosis in various tissues.[11] Cdk2 was found to regulate apoptosis in thymocytes,[12] while subcellular localisation of Cdk2 have been found as a determinant of the apoptotic or proliferative fate of mesangial cells.[13]

Cdk5 has an essential role in the central and peripheral nervous system.[14] Cdk5 regulates the cytoarchitecture of the developing brain[15] and mediates the neuronal migration in the post mitotic neurons. Cdk5 has also many important functions in the neuronal cytoskeleton dynamics, synaptic plasticity,[16] drug addiction,[17] synaptic endocytosis[18] and neurotransmitter release.[19] Cdk5 may be involved in the pathogenesis of several neurodegenerative disorders such as Alzheimer's and Parkinson's disease.[14] It has been shown that Cdk9 induces the differentiation in distinct tissues and the degree of expression of Cdk9 correlates with differentiation of primary neuroectodermal and neuroblastoma tumours.[20]

Due to the important role of Cdks in several diseases including cancer, intensive efforts to find selective cyclin-dependent kinase inhibitors (Cdki) have been made during the last 15 years. Several classes of different Cdki have been identified. In spite of their chemical diversity they share common characteristics:[21] 1) Cdkis have low molecular weights (about 600); 2) Cdkis are flat hydrophobic heterocyclic compounds; 3) Cdkis act by competing with adenosine triphosphate (ATP) for binding in the kinase ATP-binding site, and 4) Cdkis bind mostly by hydrophobic interactions and hydrogen bonds with the kinase.

Cdki have been classified according to their selectivity into three groups: 1) Pan-Cdki that inhibit Cdk1, 2, 4, 5, 6, 7 and 9 with almost similar potency like flavopiridol; 2) Selective Cdki for Cdks 1, 2, 5, 7 and 9 such as the 2, 6, 9 tri-substituted purines (olomoucine, roscovitine and purvalanol), and 3) Selective Cdki for Cdk4 and 6 (PD-0332991 or P-276–00).

(R)-Roscovitine (CYC202)

Roscovitine belongs to the 2, 6, 9 tri-substituted purines [Figure 1].[22,23] Roscovitine was found to be a selective inhibitor for Cdk1, 2, 5, 7 and 9. In the kinase inhibitory assay, roscovitine has been shown to inhibit these kinases with the IC50 at the nanomolar range.[24,25] Roscovitine was also found to inhibit several other kinases such as CaM Kinase 2, CK1α, CK1δ, DYRK1A, EPHB2, ERK1, ERK2, FAK, and IRAK4 at the micromolar range (1-40 μM). However, other kinases including Cdk4, Cdk6 and Cdk8 were not sensitive to roscovitine.[24,25]

Since Cdks have an important role in a wide range of cellular functions, roscovitine has been suggested as a potential treatment for several pathophysiologically different diseases. The effects of roscovitine have been studied *in vitro* in cell lines and *in vivo* in animal models. The *in vitro* effects of roscovitine have been studied in more than 100 cell lines. Several studies have reported the IC50 required to inhibit cell proliferation including the NCI 60 cell line panel (average IC50 = 16 μM),[23] the McClue *et al.* panel (19 cell lines; average IC50 = 15.2 μM),[26] and the Raynaud *et al.* panel (24 cell lines; average IC50 = 14.6 μM).[27] The IC50 average required for inhibition of cell proliferation in cancer cell lines does not exceed 17 μM; moreover, roscovitine was shown to be cell cycle phase non-specific. Direct inhibition of several Cdks results in inhibition of the exit from G0 (Cdk3/cyclin C), G1/S transition (Cdk2/cyclin E), S phase progression (Cdk2/ cyclin A), G2 phase (Cdk1/Cyclin A) and G2/M transition (Cdk1/Cyclin B). Depending on the cycling status of the cells, the antimitotic effects of roscovitine may comprise combinations of these mechanisms

Indirect inhibition of the cell cycle by roscovitine is mediated through the inhibition of the activity of Cdk7/ cyclin H/ MAT1 (CAK) resulting in prevention of the phosphorylation of the T loop threonine of various Cdks. This finally decreases the activity of Cdk1, 2 and 4. Also phosphorylation of the natural inhibitor p27 by Cdk2 will be diminished[28] [28] leading to its stabilisation and more inhibition of the cell cycle.[29] In addition, roscovitine was shown to inhibit the initiation of DNA synthesis,[30] the formation of centrosomes[31] and the formation of the nucleolus.[32]

Roscovitine has been shown to induce apoptosis in several cell lines regardless of the p53 status; however, roscovitine has a higher potency to induce apoptosis in wild type p53 cells compared to p53 null cells [23, 26, 33-34]. Cell death has been detected in all phases of the cell cycle and different mechanisms may be involved including inhibition of the cell cycle due to p53 activation and inhibition of Cdk7/Cdk9-dependent transcription inhibiting RNA polymerase II enzyme.[33,34] Effects of roscovitine on global transcription have been shown to be limited and only few proteins such as Mcl-1, XIAP, and survivin have been found to be severely reduced. Induction of cell death by roscovitine, thus, seems to correlate rather well with inhibition of transcription of essential cell survival factors.[35,36] Down regulation of survivin and XIAP by roscovitine was shown to contribute to the activation of caspases in glioma cell.[37] Alvi *et al.* have reported that roscovitine induced apoptotic cell death in chronic lymphocytic leukaemia B-lymphocytes at significantly higher level than in normal blood mononuclear cells, purified B- or T-lymphocytes. Apoptosis was caspase-dependent but p53- independent and was accompanied with down regulation of Mcl-1 and XIA.[38]

Anti-proliferative and pro-apoptotic effects of roscovitine have been implicated in cancer treatment and used in studies on the antitumour effects of roscovitine. So far, no cell line resistant to roscovitine has been reported until now.[39]

Interestingly, tumour cells are more dependent on the short-lived survival factors compared to normal cells, and thus, down regulation of these factors by roscovitine treatment has a higher impact on tumour cells.[40] Synergistic effects of roscovitine in combination with other chemotherapeutic agents such as camptothecin in MCF-7 breast tumour,[41] irinotecan in p53-mutated colon cancer,[42] histone deacetylase (LAQ824) in HL60 and Jurkat leukaemic cells and doxorubicin in sarcoma cell lines [43] have been shown *in vitro*.

Antitumour effects of roscovitine as a single treatment, or in combination with conventional cytostatics, have been studied *in vivo* in various tumour xenografts models. Nude mice bearing human colorectal cancer or human uterine cancer xenografts were treated with roscovitine at different dosing schedules. Roscovitine inhibited the tumour growth rate and reduced tumour volumes and weights.[26,44] Roscovitine was also shown to be effective in reducing the growth of A4573 (Ewing's sarcoma) and PC3 prostate tumour xenografts.[45.46]

The efficacy of roscovitine in non-nude BDF1 male mice bearing Glasgow osteosarcoma xenografts was investigated in relation to biological circadian rhythm. Roscovitine was administered orally (300 mg/kg x1 daily) for 5 days Zeitgeber time 3 (ZT3, 3 hours after light onset) or at ZT11 or ZT19. Roscovitine reduced the tumour growth by 35% when administered in the active time of the mice (ZT19) and 55% when administered during their rest span (ZT3 or ZT11).[47]

Roscovitine showed higher antitumour activity when combined with other antitumour treatments. Maggiorella *et al.* have reported better reduction in tumour volume from 54% to 72% when a single dose of 100 mg/kg was given intraperitoneal (i.p.) and combined with radiation therapy in mice bearing MDA-MB 231 (breast cancer).[48] Roscovitine was shown to have a synergistic effect in inhibiting HT29 colon cancer xenografts when combined with irinotecan.[42]

Pharmacokinetics and Metabolism of Roscovitine

The PK of roscovitine have been reported in mice, rats and human. Vita *et al.* reported the PK and biodistribution of roscovitine in rats after a dose of 25 mg/kg. Roscovitine PK was described by a two-compartment open model and short elimination half-life (<30 min). The highest distribution of roscovitine was observed in lungs followed by liver, fat and kidney, while exposure to roscovitine in brain was 30% of that observed in plasma. Three major metabolites were detected in plasma, but no metabolites were detected in brain.[49 50]

The PK of roscovitine was investigated in BALB/c and Tg26 mice. These studies showed rapid and biphasic clearance of roscovitine from plasma following intravenous (i.v.), i.p. or oral administration.[44,51,52] Roscovitine had rapid tissue distribution and rapid elimination with a half-life of 1.19 hr. Plasma concentrations above 15 μM (the average IC50 values obtained with various tumour cell lines) were observed for 4, 12, and 24 h following oral administration of 50, 500, and 2000 mg/kg, respectively.[44]

The PK of roscovitine in humans were reported in two phaseI trials. Roscovitine was administered orally as a single dose (50 to 800 mg) to healthy volunteers and the concentrations of roscovitine and its carboxylated metabolite were followed in plasma and urine. Roscovitine was found to undergo slow absorption from the gastrointestinal tract, but food intake did not affect the bioavailability of the drug. Roscovitine was found to have rapid metabolism and non-saturated high protein binding.[53]

In the second investigation, twenty-one patients with a median age of 62 years (range: 39–73 years) were treated with roscovitine in doses of 100, 200 and 800 mg twice daily for 7 days. The elimination half-life ranged between 2–5 hrs depending on the dose of roscovitine. Neither objective tumour responses, nor inhibition of retinoblastoma protein phosphorylation (suggested as a suitable PD endpoint) in peripheral blood mononuclear cells were observed.[54] High protein binding of roscovitine (92% to 96%) was shown in human and mice plasma.[44,55]

In vitro and *in vivo* metabolism of roscovitine was reported recently.[50,52,56,57] Several metabolites were indentified including the carboxylate metabolite (oxidation of the alcohol group at C2 of the purine ring).[50] CYP3A4 and CYP2B6 enzymes have been shown to be the main enzymes in roscovitine metabolism. Roscovitine was found to undergo phase II metabolism through conjugation with glucoronic acid by the phase II UGT1A3, 1A9 and 2B7. Moreover, roscovitine was able to inhibit its own metabolism *in vitro* through inhibition of CYP3A4 with the IC50 of 3.2 μM. Thus, possible drug-drug interactions should be considered in the clinic.[57]

PK/PD of Roscovitine in the Bone Marrow in Mice

Myelosuppression is one of the most frequent complications and a dose limiting factor for the majority of conventional chemotherapeutic agents. Depending on the dose, several cytostatics may induce complete myeloablation of the bone marrow. Studies on haematotoxicity *in vitro* and in animal models help to predict the possible side effects prior to clinical trials.

In order to investigate the myelosuppressive potential of roscovitine we studied the effect of roscovitine on bone marrow cells *in vitro* and *in vivo* in Balb/c mouse. Crude bone marrow was incubated *in vitro* with roscovitine at concentrations of 25–250 μM for 4 hrs and viability was studied using resazurin assay. The viability of bone marrow cells was decreased in a concentration-dependent manner. Concentration of 250 μM significantly reduced the viability of the cells to 70% compared to controls ($P = 0.015$) while lower concentrations did not have a significant effect. Our results were in agreement with the findings that roscovitine induced apoptosis of mature neutrophils,[58] eosinophils[59] and proliferating T-cells[60] in a concentration and exposure-time dependent manner. The myelosuppressive effect of roscovitine on haematopoietic progenitors was studied using a clonogeneic assay. Bone marrow cells were exposed to roscovitine at different concentrations (25–100 μM) for up to 24 hrs in suspension cultures. After washing, the capacity of haematopoietic progenitors to form colony-forming unit granulocyte/macrophade (CFU-GM), burst-forming unit erythroid (BFU-E) and colony-forming unit granulocyte/erythrocyte/macrophage/megakaryocyte (CFU-GEMM) colonies was studied using semisolid media. The clonogenic capacity of the bone marrow decreased in a dose- and time-dependent manner. BFU-E colonies were more sensitive than CFU-GM and completely blocked after 12 and 24 hr incubation with both 50 and 100 μM of roscovitine. Since a decrease in the colony formation in controls after 12 and 24 hrs was observed, which most probably is due to lack of the growth factors in the suspension media, bone marrow cells were exposed to roscovitine (1 - 100 μM) in semisolid MethoCult media containing growth factors. Suppression of

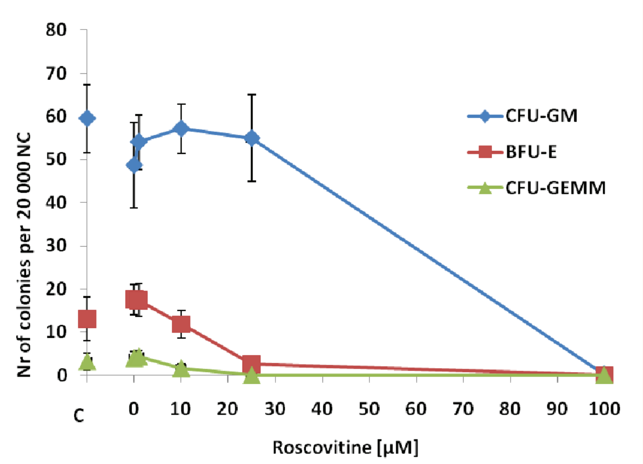

Figure 2: Effect of roscovitine on haematopoietic progenitors in vitro. 20,000 nucleated bone marrow cells were cultured in semi solid media (MethoCult M3434) with roscovitine at final concentrations of 1,10, 25 or 100 µM or dimetylsulfoxid (DMSO) (control for solvent toxicity). Colony forming unit granulocyte-macrophage (CFU-GM), erythroid-brust froming units (BFU-E) and colony-forming unit granulocyte erythrocyte monocyte macrophage (CFU-GEMM) were counted at day 12. Results are expressed as mean ± standard deviation (SD) from 5 mice.

colony formation in a concentration- and cell type-dependent manner was observed. CFU-GEMM were most sensitive and were completely blocked at 25 µM concentration, followed by BFU-E which were also significantly inhibited at 25 µM while CFU-GM were least sensitive and were inhibited at 100 µM only [Figure 2].

We further studied the myelosuppressive effect of roscovitine *in vivo* in female Balb/c mice. Roscovitine was administered to the mice and bone marrow cells were cultured in the MethoCult media and assessed for colonogenic growth. No myelosuppressive effect was detected after the administration of a single dose of roscovitine up to 250 mg/kg. Then, roscovitine was administered at a dose of 175 mg/kg twice daily for 4 days. Only transient inhibition of the BFU-E colonies occurred one day after the last dose of roscovitine. The colony formation capacity of bone marrow was recovered 5 days after the last dose of roscovitine [Figure 3]. The lack of activity of roscovitine on haematopoietic progenitors *in vivo* was not expected after its proven inhibitory effect *in vitro* and the reported activity on different xenografts *in vivo.*[26,44,46,47] Therefore we decided to study the distribution and PK of roscovitine in Balb/c mice. Roscovitine was administered as a single i.p. injection in a dose of 50 mg/kg. As presented in Table 1, roscovitine had a short half-life (less than 1 hr) and only a small fraction of roscovitine (about 1.5%) reached the bone marrow compared to plasma. Thus, low distribution of roscovitine to bone marrow may explain the low haematotoxicity *in vivo*. This example illustrates the importance of PK/PD and biodistribution in preclinical studies. This may be also implicated in the fact that, despite a good cytotoxic effect of roscovitine in leukaemic cell lines in vitro, the therapeutic potential of roscovitine in haematological malignancies may be limited.

Age-Dependent Kinetics and Dynamics of Roscovitine in Rat Brains

Age-dependent PK is an important issue when the drug may be used in the treatment of paediatric patients and/or when the drug has a narrow therapeutic window. Unfortunately, scaling down the PK data from adults to paediatrics, has been proven not to be sufficiently predictive for many drugs.[4] Roscovitine has been found to inhibit different solid and haematological tumour cell lines including acute lymphoblastic leukaemia (ALL)[61] which is frequent in children and is correlated with a high central nervous system (CNS) relapse rate.[62]

Recently, we have explored the effect of age on the PK of roscovitine and investigated the effect of roscovitine on two neuronal targets, Cdk5 and Erk1/2, in different brain regions.[63] Fourteen day-old pups and adult Sprague-Dawley rats were

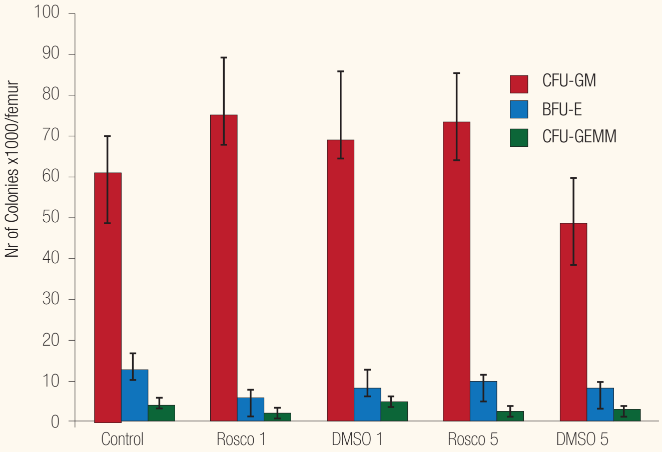

Figure 3: Effect of roscovitine on haematopoietic progenitors in vivo. Mice were treated withroscovitine (350 mg/kg/day) divided into two daily doses for 4 days. Mice treated with dimetylsulfoxid (DMSO) and untreated animals served as controls. Bone marrow was examined 1 and 5 days after the last dose of roscovitine. Colony forming unit granulocyte-macrophage (CFU-GM), erythroid-brust froming units (BFU-E) and colony-forming unit granulocyte erythrocyte monocyte macrophage (CFU-GEMM) were counted on day 12. Each group consisted of 5 mice, control group of 8 mice. Results are expressed as mean ± standard deviation (SD).

treated with a single i.p. injection of roscovitine in a dose of 25 mg/kg and plasma and brain were sampled at different time points. Table 2 shows the pharmacokinetic parameters of roscovitine in plasma and in different brain regions in pups and adult rats. The PK of roscovitine was best described by a 2-compartment open model with distribution half-lives of 0.6 hrs in pups and 0.06 hr in adult rats. A significantly longer elimination half-life (7 hrs) was observed in the plasma and brain of the rat pups compared to 30 and 20 min found in the plasma and brain in adult rats, respectively.

The area under the concentration–time curve (AUC) of roscovitine was 22-fold higher in the pups' plasma and 100-fold higher in the pups' brains compared to that found in adult rats [Figure 4]. No significant difference between roscovitine AUC in plasma and AUCs in different brain regions in pups was found. On the contrary, in adult rats, the AUC of roscovitine in the brain was about 25% of that found in plasma (Table 2). The C_{max} was significantly ($P < 0.05$) higher (>22 µg/g) in pups brain compared to that found in plasma, while 4-fold higher C_{max} was found in plasma compared to that observed the brain (17.7 µg/ml and about 4 µg/g, respectively) in adult rats. The high concentrations of roscovitine found in the pups' brains indicate the free passage of roscovitine into the brain.

This difference in exposure might be due to the immaturity of the CYP450 enzymes responsible for roscovitine metabolism[64] or immaturity of BBB. Roscovitine is metabolised in humans mainly by CYP3A4 and CYP2B6 enzymes.[57] Several CYP450 enzymes are not fully matured at the age of 2 weeks in rats.[65] A similar situation was also reported in humans and CYP3A4, for example, approaches the adult full capacity only after first year of life.[66,67]

Most chemotherapeutic agents do not cross the BBB and do not reach the CNS in enough high concentrations to eliminate tumour cells despite

Table 1: Pharmacokinetic parameters in plasma and bone marrow following intraperitoneal administration of roscovitine (50 mg/kg)

	AUC µmol/l .h	C_{max} µmol/l	Cl l/h	V^d l	T½ h
Plasma	275.8	202	0.05	0.015	0.82
BM	4.6	4.9	0.62	0.54	0.61

Legend: AUC = area under the concentration–time curve (AUC is derived using WinNonlin analysis); (C_{max}) = estimated maximum concentrations; Cl = clearance; V_d = apparent volume of distribution; T½ = half-life; BM = Bone marrow.

Table 2: Pharmacokinetic parameters in plasma and brain of adult and pups rats. Results are presented as mean ± standard deviation (SD) (n = 3)

PK parameters		Plasma	Frontal Cortex	Hippocampus	Cerebellum
AUC (h.µg/ml)/ (h.µg/g)	Pups	66.79 ± 7.15	69.57 ± 15	74.92 ± 12	78.72 ± 11.2
	Adults	3.01 ± 0.21	0.71 ± 0.14	0.58 ± 0.03	0.62 ± 0.06
Tα (h)	Pups	0.50 ± 0.09	0.48 ± 0.19	0.43 ± 0.1	0.59 ± 0.14
	Adults	0.081 ± 0.05	0.045 ± 0.02	0.062 ± 0.012	0.062 ± 0.018
Tβ (h)	Pups	7.2 ± 1.4	6.8 ± 1.3	8.0 ± 1.7	7.7 ± 2.2
	Adults	0.54 ± 0.26	0.35 ± 0.13	0.36 ± 0.15	0.42 ± 0.18
C_{max} (µg/ml)/ (µg/g)	Pups	15.79 ± 0.38	24.9 ± 1.8	24.75 ± 1.9	23.69 ± 1.4
	Adults	17.71 ± 4.42	4.47 ± 0.70	4.64 ± 0.81	3.81 ± 1.22
Vss (ml)	Pups	88 ± 15.3	90 ± 21	86 ± 20	102 ± 13
	Adults	650 ± 223	1095 ± 167	2056 ± 219	1909 ± 484
Cl (ml/h)	Pups	9.7 ± 1.2	10.2 ± 1.5	11.1 ± 2	11.3 ± 1.2
	Adults	1637 ± 118	7262 ± 1612	8737 ± 452	8139 ± 727

Legend: AUC = Area under the concentration–time curve; Tα,Tβ = distribution and elimination half-lives; C_{max} *= maximum concentration; Vss = volume of distribution; Cl = clearance.*

the high systemic exposure. Roscovitine was highly distributed over the BBB in the pups and the brain exposure in all studied regions (e.g. hippocampus, cerebral cortex and cerebellum) was 100% of that found in plasma which can be compared to about 25% that has been found in the brain of adult rats. The high distribution to the brain could be explained by an age-dependent variation in the maturity and function of BBB. Butt *et al.* have shown that the BBB of the rat fully matures 3–4 weeks postnatal.[68] No roscovitine metabolites were found in the brains of both adult and young rats.

In pups, roscovitine concentrations in plasma and brain were higher than the reported IC50 (10-15 µM) for cancer cell lines for more than 8 hours. However, this level of exposure was achieved for less

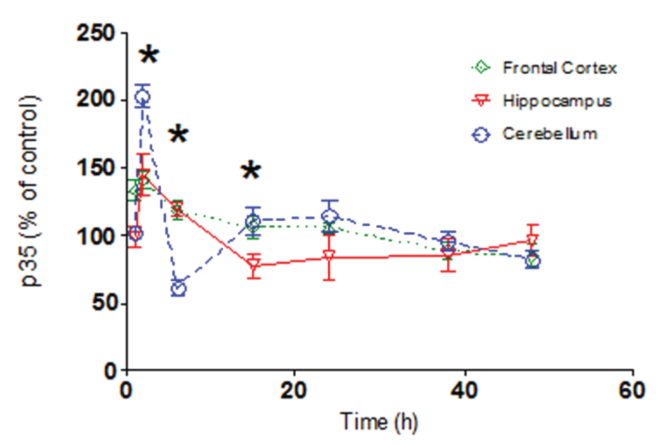

Figure 5: Effect of roscovitine on cyclin-dependent kinase 5 – neuronal protein specific cyclin-dependent kinase (Cdk) Cdk5 regulator (Cdk5-p35) in different brain parts of 14 days old rat pups after single intraperitoneal (i.p.) injection of 25 mg/kg. Pups were killed at different time points after injection, brains dissected, homogenised, and immunoblotted for active Cdk5-p35. The figure shows densitometric analysis of the Western blotting bands for both p35 in the frontal cortex, hippocampus and cerebellum until 48 hr after single i.p. injection of roscovitine. Data are presented as mean ± standard deviation (SD) of values expressed as percentage of control animals (*, $P < 0.05$ for analysis of p35 data; Analysis of variance (ANOVA) followed by all pairwise Fisher's Protected Least Significant Difference (PLSD) testing were used.

than 30 minutes in plasma and brain of adult rats. These results may be implicated in the treatment of paediatric malignancies especially brain tumours.

Roscovitine is a potent inhibitor of Cdk5 which has important function in the developing brain such as neuronal migration.[15] Moreover, the negative feedback regulation of mitogen activated protein kinases (MAPK) signalling by Cdk5 has been suggested to be important for neuronal survival.[69]

High concentrations of roscovitine found in the brain of pups raised the question about the effects of roscovitine on target enzymes. We assessed the expression of p35 as an indicator of Cdk5 activity. Inhibition of p35 phosphorylation by Cdk5 stabilises it and delays its proteasomal degradation.[70,71] Roscovitine induced a transient and significant accumulation of p35 protein in all brain regions in rat pups that indicates the inhibition of the Cdk5 enzyme. An increase in p35 was found in the frontal cortex 1–2 hrs post-administration (140% of controls, Figure 5, $P < 0.05$), in the hippocampus and in cerebellum at 2 hrs post-administration (150% and 200%, respectively, Figure 5). The levels of p35 were normalised at 6–15 h [Figure 5]. No change in p35 levels was observed in the adult brain which probably is due to the low concentration and the rapid elimination half-life.

Cdk5 was found to inhibit Erk1/2 phosphorylation by a MEK1 and RasGRF2 mediated mechanism and the inhibition of Cdk5 by roscovitine increased the levels of phosphorylated Erk1/2 (active form) in neuronal cells *in vitro*.[69,72] At early time points after administration of roscovitine, the accumulation of p35 protein was accompanied by increased levels of the phosphorylated (activated) form of Erk1/2. In the frontal cortex and hippocampus, a transient activation of Erk1/2 was observed at 1 and 2 hrs after injection [Figure 6]. In the cerebellum, significant increases of pErk1/2 levels at 2 hrs were followed by a significant decrease at 6 hrs after administration [Figure 6]. At later time points, levels of pErk1/2 returned to control levels in all brain regions [Figure 6]. Altogether, roscovitine was presented in the brain of rat pups in sufficient amounts to inhibit the Cdk5 resulting in increased phosphorylation of Erk1/2.

Discussion

Cyclin-dependent kinases (Cdks) are serine/threonine kinases that play key roles in cell cycle progression and RNA transcription. Deregulation of Cdks has been shown in several diseases including several types of cancer in which increased activity of Cdks has been observed. Synthetic cyclin dependent kinase inhibitors (Cdkis) are small heterocyclic compounds which compete

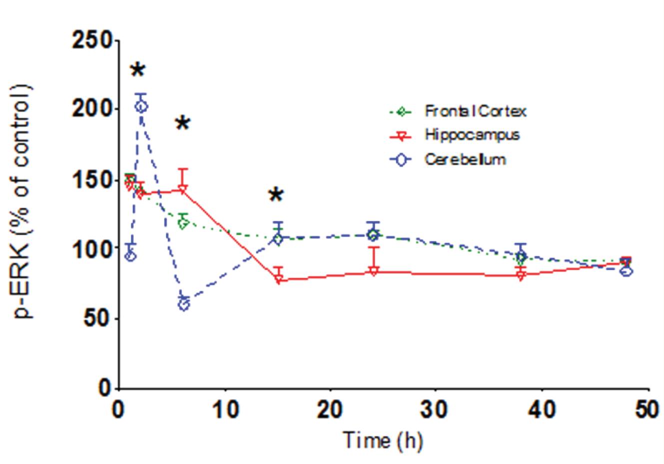

Figure 6: Effect of roscovitine on p-Erk in different brain parts of rat pups 14 days old after single intrapertoneal (i.p.) injection of 25 mg/kg. Pups were killed at different time points after injection, brains dissected, homogenized, and immunoblotted for active phosphorylated Erk1/2. Control animals were injected with vehicle. The figure show densitometric analysis of the Western blotting bands for pErk1/2 in the frontal cortex, hippocampus and cerebellum until 48 hr after single i.p. injection of roscovitine. Data are presented as mean ± standard deviation (SD) of values expressed as percentage of control animals (*, $P < 0.05$ is the significant level for analysis of p-ERK data; Analysis of variance (ANOVA) followed by all pairwise Fisher's Protected Least Significant Difference (PLSD) post-hoc test).

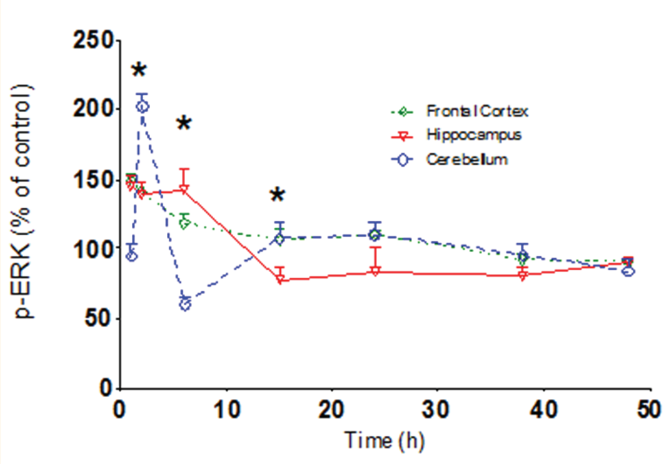

Figure 6: Effect of roscovitine on p-Erk in different brain parts of rat pups 14 days old after single intrapertoneal (i.p.) injection of 25 mg/kg. Pups were killed at different time points after injection, brains dissected, homogenized, and immunoblotted for active phosphorylated Erk1/2. Control animals were injected with vehicle. The figure show densitometric analysis of the Western blotting bands for pErk1/2 in the frontal cortex, hippocampus and cerebellum until 48 hr after single i.p. injection of roscovitine. Data are presented as mean ± standard deviation (SD) of values expressed as percentage of control animals (*, $P < 0.05$ is the significant level for analysis of p-ERK data; Analysis of variance (ANOVA) followed by all pairwise Fisher's Protected Least Significant Difference (PLSD) post-hoc test).

with ATP and inhibit the phosphorylation of the target substrates. Exposure of tumour cells to Cdkis results in both cell cycle arrest and apoptosis.

The family of 2,6,9-trisubstituted purines are one of the first described Cdk inhibitors.[73] The (R)-stereoisomer of roscovitine is a member of this family and has now reached phaseII clinical trials for non-small cell lung (NSCL) cancer and nasopharyngeal cancers and phaseI trials for glomerulonephritis. Preclinical investigations of the role of roscovitine in the treatment of neurodegenerative disorders such as Alzheimer's disease, viral infections, protozoal infections and inflammatory diseases are ongoing. Roscovitine has a rapid metabolism and short elimination half-life in rodents and man.[44,50,52,53] The poor pharmacokinetic profile and the insufficient exposure to the drug in cancer patients may explain the modest success in the clinical trials.[54] Current research is focusing on overcoming pharmacokinetic barriers that limit the clinical use of roscovitine. Moreover, a novel class of second generation analogues of roscovitine has been designed and is under development. Studies on the pan-Cdk inhibitor flavopiridol confirmed the importance of optimising the schedule of dosing according to the PK/PD relationship. By changing the dose schedule from 72 hrs infusion to 30 minutes i.v. bolus followed by a 4-hrs infusion, a significant difference in the clinical outcome and final response of refractory CLL patients was achieved.[74]

No myelosuppression has been reported until now in the preclinical and clinical studies with roscovitine.[51,54] However, clinically beneficial low haematotoxicity of roscovitine may reflect in reality poor distribution of roscovitine to the bone marrow. *In vitro*, the haematopoietic progenitors were inhibited by roscovitine within the same exposure range as the tumour cells when comparing the inhibitory AUC reported for tumour cell lines [26,44] with the inhibitory AUC of the haematopoietic progenitors found in our study.

Under certain circumstances the haematotoxicity of roscovitine may become more evident: 1) Changes in the form of administration, aiming to increase the half-life of the drug, may result in higher exposure to roscovitine and changes in biodistribution. This in turn may change the toxicity profile; 2) A combination of roscovitine with radiation therapy, which increases the permeability of blood-bone marrow barrier,[75] and thus the distribution of some drugs to the bone marrow, may increase the myelotoxicity of roscovitine, and 3) In pediatric patients where age-dependent longer elimination half-life is most likely leading to higher exposure of haematopoietic progenitors to roscovitine and thus toxicity risk.[66]

Age dependent PK is an important issue concerning toxic drugs and drugs with a narrow therapeutic window such as anticancer drugs, where underdosing may lead to relapse while overdosing can cause severe side effects. Age dependent kinetics were reported for several drugs including cis platin, busulfan, thioguanine, etoposide, lamivudine and mycophenolate mofetil.[76-81] Our studies showed that roscovitine elimination half-life was 14-fold higher in young rats compared to adults. Moreover, the exposure to the drug was 22-fold and 100-fold higher in the plasma and brain, respectively. These results indicate the importance of early determination of the PK-parameters in different age groups.

Conclusion

Roscovitine inhibits mouse haematopoietic progenitors *in vitro* within the same concentration range required to inhibit malignant cells; however, the cytotoxic effect of roscovitine on haematopoietic progenitors *in vivo* is transient due to a short half-life in combination with low distribution to the arrow compartment.

Roscovitine demonstrates age-dependent PK. Prolonged systemic and brain exposure to roscovitine was found in pups compared to adult rats, which may be due to immature CYP450 enzymes as well as the BBB. Moreover, roscovitine was able to induce a transient effect on critical neuronal targets and signalling pathways in the brain of young rats. These studies show the importance of early pharmacokinetic and phamacodynamic studies in drug development.

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References

1. Lin JH, Lu AY. Role of pharmacokinetics and metabolism in drug discovery and development. Pharmacol Rev 1997; 49:403–49.
2. Meibohm B, Derendorf H. Pharmacokinetic/pharmacodynamic studies in drug product development. J Pharm Sci 2002; 91:18–31.
3. Holford NH. Target concentration intervention: Beyond Y2K. Br J Clin Pharmacol 2001; 52:55–9.
4. Bjorkman S. Prediction of cytochrome p450-mediated hepatic drug clearance in neonates, infants and children:How accurate are available scaling methods? Clin Pharmacokinet 2006; 45:1–11.
5. Bjorkman S. Prediction of drug disposition in infants and children by means of physiologically based pharmacokinetic (PBPK) modelling: Theophylline and midazolam as model drugs. Br J Clin Pharmacol 2005; 59:691–704.
6. Domingo JL. Cobalt in the environment and its toxicological implications. Rev Environ Contam Toxicol 1989; 108:105–32.
7. Malumbres M, Barbacid M. Mammalian cyclin-dependent kinases. Trends Biochem Sci 2005; 30:630–41.
8. Perez de Castro I, de Carcer G, Malumbres M. A census of mitotic cancer genes: New insights into tumor cell biology and cancer therapy. Carcinogenesis 2007; 28:899–912.
9. Strock CJ, Park JI, Nakakura EK, Bova GS, Isaacs JT, Ball DW, et al. Cyclin-dependent kinase 5 activity controls cell motility and metastatic potential of prostate cancer cells. Cancer Res 2006; 66:7509–15.
10. Goodyear S, Sharma MC. Roscovitine regulates invasive breast cancer cell (MDA-MB231) proliferation and survival through cell cycle regulatory protein cdk5. Exp Mol Pathol 2007; 82:25–32.
11. Zhang Q, Ahuja HS, Zakeri ZF, Wolgemuth DJ. Cyclin-dependent kinase 5 is associated with apoptotic cell death during development and tissue remodeling. Dev Biol 1997; 183:222–33.
12. Hakem A, Sasaki T, Kozieradzki I, Penninger JM. The cyclin-dependent kinase Cdk2 regulates thymocyte apoptosis. J Exp Med 1999; 189:957–68.
13. Hiromura K, Pippin JW, Blonski MJ, Roberts JM, Shankland SJ. The subcellular localization of cyclin dependent kinase 2 determines the fate of mesangial cells: Role in apoptosis and proliferation. Oncogene 2002; 21:1750–8.
14. Cruz JC, Tsai LH. A Jekyll and Hyde kinase: Roles for Cdk5 in brain development and disease. Curr Opin Neurobiol 2004; 14:390–4.
15. Dhavan R, Tsai LH. A decade of CDK5. Nat Rev Mol Cell Biol 2001; 2:749–59.
16. Angelo M, Plattner F, Giese KP. Cyclin-dependent kinase 5 in synaptic plasticity, learning and memory. J Neurochem 2006; 99:353–70.
17. Bibb JA. Role of Cdk5 in neuronal signaling, plasticity, and drug abuse. Neurosignals 2003; 12:191–9.
18. Nguyen C, Bibb JA. Cdk5 and the mystery of synaptic vesicle endocytosis. J Cell Biol 2003; 163:697–9.
19. Tomizawa K, Ohta J, Matsushita M, Moriwaki A, Li ST, Takei K, et al. Cdk5/p35 regulates

neurotransmitter release through phosphorylation and downregulation of P/Q-type voltage-dependent calcium channel activity. J Neurosci 2002; 22:2590–7.

20. Canduri F, Perez PC, Caceres RA, de Azevedo WF, Jr. CDK9 a potential target for drug development. Med Chem 2008; 4:210–8.
21. Knockaert M, Greengard P, Meijer L. Pharmacological inhibitors of cyclin-dependent kinases. Trends Pharmacol Sci 2002; 23:417–25.
22. De Azevedo WF, Leclerc S, Meijer L, Havlicek L, Strnad M, Kim SH. Inhibition of cyclin-dependent kinases by purine analogues: Crystal structure of human cdk2 complexed with roscovitine. Eur J Biochem 1997; 243:518–26.
23. Meijer L, Borgne A, Mulner O, Chong JP, Blow JJ, Inagaki N, et al. Biochemical and cellular effects of roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdc2, cdk2 and cdk5. Eur J Biochem 1997; 243:527–36.
24. Bach S, Knockaert M, Reinhardt J, Lozach O, Schmitt S, Baratte B, et al. Roscovitine targets, protein kinases and pyridoxal kinase. J Biol Chem 2005; 280:31208–19.
25. Tang L, Li MH, Cao P, Wang F, Chang WR, Bach S, et al. Crystal structure of pyridoxal kinase in complex with roscovitine and derivatives. J Biol Chem 2005; 280:31220–9.
26. McClue SJ, Blake D, Clarke R, Cowan A, Cummings L, Fischer PM, et al. In vitro and in vivo antitumor properties of the cyclin dependent kinase inhibitor CYC202 (R-roscovitine). Int J Cancer 2002; 102:463–8.
27. Raynaud FI, Fischer PM, Nutley BP, Goddard PM, Lane DP, Workman P. Cassette dosing pharmacokinetics of a library of 2,6,9-trisubstituted purine cyclin-dependent kinase 2 inhibitors prepared by parallel synthesis. Mol Cancer Ther 2004; 3:353–62.
28. Bloom J, Pagano M. Deregulated degradation of the cdk inhibitor p27 and malignant transformation. Semin Cancer Biol 2003; 13:41–7.
29. Zhang GJ, Safran M, Wei W, Sorensen E, Lassota P, Zhelev N, et al. Bioluminescent imaging of Cdk2 inhibition in vivo. Nat Med 2004; 10:643–8.
30. Krude T. Initiation of human DNA replication in vitro using nuclei from cells arrested at an initiation-competent state. J Biol Chem 2000; 275:13699–707.
31. Matsumoto Y, Hayashi K, Nishida E. Cyclin-dependent kinase 2 (Cdk2) is required for centrosome duplication in mammalian cells. Curr Biol 1999; 9:429–32.
32. Sirri V, Hernandez-Verdun D, Roussel P. Cyclin-dependent kinases govern formation and maintenance of the nucleolus. J Cell Biol 2002; 15:969–81.
33. Wesierska-Gadek J, Gueorguieva M, Horky M. Roscovitine-induced up-regulation of p53AIP1 protein precedes the onset of apoptosis in human MCF-7 breast cancer cells. Mol Cancer Ther 2005; 4:113–24.
34. Wesierska-Gadek J, Wandl S, Kramer MP, Pickem C, Krystof V, Hajek SB. Roscovitine up-regulates p53 protein and induces apoptosis in human HeLaS(3) cervix carcinoma cells. J Cell Biochem 2008; 105:1161–71.
35. Lacrima K, Valentini A, Lambertini C, Taborelli M, Rinaldi A, Zucca E, et al. In vitro activity of cyclin-dependent kinase inhibitor CYC202 (Seliciclib, R-roscovitine) in mantle cell lymphomas. Ann Oncol 2005; 16:1169–76.
36. Mohapatra S, Chu B, Zhao X, Pledger WJ. Accumulation of p53 and reductions in XIAP abundance promote the apoptosis of prostate cancer cells. Cancer Res 2005; 65:7717–23.
37. Kim EH, Kim SU, Shin DY, Choi KS. Roscovitine sensitizes glioma cells to TRAIL-mediated apoptosis by downregulation of survivin and XIAP. Oncogene 2004; 23:446–56.
38. Alvi AJ, Austen B, Weston VJ, Fegan C, MacCallum D, Gianella-Borradori A, et al. A novel CDK inhibitor, CYC202 (R-roscovitine), overcomes the defect in p53-dependent apoptosis in B-CLL by down-regulation of genes involved in transcription regulation and survival. Blood 2005; 105:4484–91.
39. Wesierska-Gadek J, Gueorguieva M, Horky M. Dual action of cyclin-dependent kinase inhibitors: Induction of cell cycle arrest and apoptosis. A comparison of the effects exerted by roscovitine and cisplatin. Pol J Pharmacol 2003; 55:895–902.
40. Meijer L, Bettayeb K, Galons H. Roscovitine (CYC202, Seliciclib). In: Smith PJ and Yue E, Eds. Monographs on Enzyme inhibitors. CDK Inhibitors and their Potential as Anti-Tumor Agents. Colchester: CRC Press, Taylor & Francis, 2006. Pp.187–226.
41. Lu W, Chen L, Peng Y, Chen J. Activation of p53 by roscovitine-mediated suppression of MDM2 expression. Oncogene 2001; 20:3206–16.
42. Abal M, Bras-Goncalves R, Judde JG, Fsihi H, De Cremoux P, Louvard D, et al. Enhanced sensitivity to irinotecan by Cdk1 inhibition in the p53-deficient HT29 human colon cancer cell line. Oncogene 2004; 23:1737–44.
43. Lambert LA, Qiao N, Hunt KK, Lambert DH, Mills GB, Meijer L, et al. Autophagy: a novel mechanism of synergistic cytotoxicity between doxorubicin and roscovitine in a sarcoma model. Cancer Res 2008; 68:7966–74.
44. Raynaud FI, Whittaker SR, Fischer PM, McClue S, Walton MI, Barrie SE, et al. In vitro and in vivo pharmacokinetic-pharmacodynamic relationships for the trisubstituted aminopurine cyclin-dependent kinase inhibitors olomoucine, bohemine and CYC202. Clin Cancer Res 2005; 11:4875–87.

45. Payton M, Chung G, Yakowec P, Wong A, Powers D, Xiong L, et al. Discovery and evaluation of dual CDK1 and CDK2 inhibitors. Cancer Res 2006; 6:4299–308.

46. Tirado OM, Mateo-Lozano S, Notario V. Roscovitine is an effective inducer of apoptosis of Ewing's sarcoma family tumor cells in vitro and in vivo. Cancer Res 2005; 65:9320–7.

47. Iurisci I, Filipski E, Reinhardt J, Bach S, Gianella-Borradori A, Iacobelli S, et al. Improved tumor control through circadian clock induction by Seliciclib, a cyclin-dependent kinase inhibitor. Cancer Res 2006; 66:10720–8.

48. Maggiorella L, Deutsch E, Frascogna V, Chavaudra N, Jeanson L, Milliat F, et al. Enhancement of radiation response by roscovitine in human breast carcinoma in vitro and in vivo. Cancer Res 2003; 63:2513–7.

49. Vita M, Meurling L, Pettersson T, Cruz-Siden M, Siden A, Hassan M. Analysis of roscovitine using novel high performance liquid chromatography and UV-detection method: pharmacokinetics of roscovitine in rat. J Pharm Biomed Anal 2004; 34:425–31.

50. Vita M, Abdel-Rehim M, Olofsson S, Hassan Z, Meurling L, Siden A, et al. Tissue distribution, pharmacokinetics and identification of roscovitine metabolites in rat. Eur J Pharm Sci 2005; 25:91–103.

51. Gherardi D, D'Agati V, Chu TH, Barnett A, Gianella-Borradori A, Gelman IH, et al. Reversal of collapsing glomerulopathy in mice with the cyclin-dependent kinase inhibitor CYC202. J Am Soc Nephrol 2004; 15:1212–22.

52. Nutley BP, Raynaud FI, Wilson SC, Fischer PM, Hayes A, Goddard PM, et al. Metabolism and pharmacokinetics of the cyclin-dependent kinase inhibitor R-roscovitine in the mouse. Mol Cancer Ther 2005; 4:125–39.

53. De la Motte S, Gianella-Borradori A. Pharmacokinetic model of R-roscovitine and its metabolite in healthy male subjects. Int J Clin Pharmacol Ther 2004; 42:232–9.

54. Benson C, White J, De Bono J, O'Donnell A, Raynaud F, Cruickshank C, et al. A phase I trial of the selective oral cyclin-dependent kinase inhibitor seliciclib (CYC202; R-Roscovitine), administered twice daily for 7 days every 21 days. Br J Cancer 2007; 96:29–37.

55. Vita M, Abdel-Rehim M, Nilsson C, Hassan Z, Skansen P, Wan H, et al. Stability, pKa and plasma protein binding of roscovitine. J Chromatogr B Analyt Technol Biomed Life Sci 2005; 821:75–80.

56. Cervenkova K, Belejova M, Chmela Z, Rypka M, Riegrova D, Michnova K, et al. In vitro glycosidation potential towards olomoucine-type cyclin-dependent kinase inhibitors in rodent and primate microsomes. Physiol Res 2003; 52:467–74.

57. McClue SJ, Stuart I. Metabolism of the trisubstituted purine cyclin-dependent kinase inhibitor seliciclib (r-roscovitine) in vitro and in vivo. Drug Metab Dispos 2008; 36:561–70.

58. Rossi AG, Sawatzky DA, Walker A, Ward C, Sheldrake TA, Riley NA, et al. Cyclin-dependent kinase inhibitors enhance the resolution of inflammation by promoting inflammatory cell apoptosis. Nat Med 2006; 12:1056–64.

59. Duffin R, Leitch AE, Sheldrake TA, Hallett JM, Meyer C, Fox S, et al. The CDK inhibitor, R-roscovitine, promotes eosinophil apoptosis by down-regulation of Mcl-1. FEBS Lett 2009; 583:2540–6.

60. Li L, Wang H, Kim J, Pihan G, Boussiotis V. The cyclin dependent kinase inhibitor (R)-roscovitine prevents alloreactive T cell clonal expansion and protects against acute GvHD. Cell Cycle 2009; 8:1794–802.

61. Yu C, Rahmani M, Dai Y, Conrad D, Krystal G, Dent P, et al. The lethal effects of pharmacological cyclin-dependent kinase inhibitors in human leukemia cells proceed through a phosphatidylinositol 3-kinase/Akt-dependent process. Cancer Res 2003; 63:1822–33.

62. Wellwood J, Taylor K. Central nervous system prophylaxis in haematological malignancies. Intern Med J 2002; 32:252–8.

63. Sallam H, Jimenez P, Song H, Vita M, Cedazo-Minguez A, Hassan M. Age-dependent pharmacokinetics and effect of roscovitine on Cdk5 and Erk1/2 in the rat brain. Pharmacol Res 2008; 58:32–7.

64. Nouws JF. Pharmacokinetics in immature animals: A review. J Anim Sci 1992; 70:3627–34.

65. Rich KJ, Boobis AR. Expression and inducibility of P450 enzymes during liver ontogeny. Microsc Res Tech 1997; 39:424–35.

66. Alcorn J, McNamara PJ. Pharmacokinetics in the newborn. Adv Drug Deliv Rev 2003; 55:667–86.

67. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology - drug disposition, action, and therapy in infants and children. N Engl J Med 2003; 349:1157–67.

68. Butt AM, Jones HC, Abbott NJ. Electrical resistance across the blood-brain barrier in anaesthetized rats: A developmental study. J Physiol 1990; 429:47–62.

69. Zheng YL, Li BS, Kanungo J, Kesavapany S, Amin N, Grant P, et al. Cdk5 Modulation of mitogen-activated protein kinase signaling regulates neuronal survival. Mol Biol Cell 2007; 18:404–13.

70. Nikolic M, Tsai LH. Activity and regulation of p35/Cdk5 kinase complex. Methods Enzymol 2000; 325:200–13.

71. Patrick GN, Zhou P, Kwon YT, Howley PM, Tsai LH. p35, the neuronal-specific activator of cyclin-dependent kinase 5 (Cdk5) is degraded by the

ubiquitin-proteasome pathway. J Biol Chem 1998; 273:24057–64.

72. Kesavapany S, Amin N, Zheng YL, Nijhara R, Jaffe H, Sihag R, et al. P35/cyclin-dependent kinase 5 phosphorylation of ras guanine nucleotide releasing factor 2 (RasGRF2) mediates rac-dependent extracellular signal-regulated kinase 1/2 activity, altering RasGRF2 and microtubule-associated protein 1b distribution in neurons. J Neurosci 2004; 24:4421–31.

73. Meijer L, Raymond E. Roscovitine and other purines as kinase inhibitors. From starfish oocytes to clinical trials. Acc Chem Res 2003; 36:417–25.

74. Phelps MA, Lin TS, Johnson AJ, Hurh E, Rozewski DM, Farley KL, et al. Clinical response and pharmacokinetics from a phase 1 study of an active dosing schedule of flavopiridol in relapsed chronic lymphocytic leukemia. Blood 2009; 113:2637–45.

75. Daldrup-Link HE, Link TM, Rummeny EJ, August C, Konemann S, Jurgens H, et al. Assessing permeability alterations of the blood-bone marrow barrier due to total body irradiation: In vivo quantification with contrast enhanced magnetic resonance imaging. Bone Marrow Transplant 2000; 25:71–8.

76. Bhatia M, Militano O, Jin Z, Figurski M, Shaw L, Moore V, et al. An age-dependent pharmacokinetic study of intravenous and oral mycophenolate mofetil in combination with tacrolimus for GVHD prophylaxis in pediatric allogeneic stem cell transplantation recipients. Biol Blood Marrow Transplant 2010; 16:333–43.

77. Burger DM, Verweel G, Rakhmanina N, Verwey-Van Wissen CP, La Porte CJ, Bergshoeff AS, et al. Age-dependent pharmacokinetics of lamivudine in HIV-infected children. Clin Pharmacol Ther 2007; 81:517–20.

78. Hassan M, Ljungman P, Bolme P, Ringden O, Syruckova Z, Bekassy A, et al. Busulfan bioavailability. Blood 1994; 84:2144–50.

79. Hassan M, Oberg G, Bekassy AN, Aschan J, Ehrsson H, Ljungman P, et al. Pharmacokinetics of high-dose busulphan in relation to age and chronopharmacology. Cancer Chemother Pharmacol 1991; 28:130–4.

80. Palle J, Frost BM, Gustafsson G, Hellebostad M, Kanerva J, Liliemark E, et al. Etoposide pharmacokinetics in children treated for acute myeloid leukemia. Anticancer Drugs 2006; 17:1087–94.

81. Palle J, Frost BM, Petersson C, Hasle H, Hellebostad M, Kanerva J, et al. Thioguanine pharmacokinetics in induction therapy of children with acute myeloid leukemia. Anticancer Drugs 2009; 20:7–14.