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Cardiotoxic effects of arsenic trioxide/imatinib mesilate combination in rats

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Abstract

Cardiotoxicity is an important consideration in the evaluation of cancer chemotherapy, because chemotherapy-induced myocardial damage might be irreversible and lethal. This in-vivo study investigated the cardiotoxicity of either arsenic trioxide or imatinib mesilate, or a combination of both drugs, following repeated administration in male Wistar rats. Both arsenic trioxide and imatinib mesilate were administered daily at a dose of 5 mg kg^{-1} intraperitoneally and 30 mg kg^{-1} orally for 10 days, respectively. Cardiotoxicity was evaluated by biochemical and histopathological examination 48 h after the last dose. Treatment with either arsenic or imatinib, or both, resulted in significant increases in serum creatine kinase isoenzyme (CK-MB), glutathione peroxidase (GPx), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) activity levels. Cardiac tissue of rats treated with arsenic showed significant increases in levels of reduced glutathione (GSH) content, GPx activity, malondialdehyde (MDA) and total nitrate/nitrite (NOx), whereas imatinib treatment significantly increased cardiac GSH content and MDA production level and decreased GPx activity level and NOx content. A combination of arsenic and imatinib produced significant increases in cardiac GSH content, GPx activity and MDA production levels, in addition to a reduction in NOx content. Combination arsenic/imatinib treatment extensively increased GPx activity and MDA production levels compared with imatinib treatment alone. Moreover, rats treated with arsenic or imatinib, or both, showed a significant increase in serum bilirubin, creatinine and urea levels. Histopathological examination of cardiac tissue of the combination-treated group revealed fibroblastic proliferation, myocardial disorganization and myocardial necrosis. Liver peroxidative alterations revealed that treatment with either arsenic or imatinib, or the two combined, increased levels of reduced-GSH and MDA production levels. However, imatinib treatment depleted liver GPx activity level contrary to treatment with the combination. Rats treated with arsenic alone or arsenic/imatinib combination showed significant elevation in liver NOx. In conclusion, both arsenic trioxide and imatinib mesilate might have significant cardiotoxicity and cardiac function should be monitored during treatment with them alone or in combination, as well as in the presence of pre-existing cardiac dysfunction.

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Introduction

Imatinib mesilate (imatinib, Glivec) is a protein kinase inhibitor used in the treatment of chronic myelogenous leukaemia (CML) and gastrointestinal stromal tumour (GIST) (Nakajima & Toga 2003). Imatinib was developed as a specific inhibitor for Bcr-Ab1 protein tyrosine kinase. Also, imatinib inhibits the receptors for platelet-derived growth factor (PDGF) and stem cell factor (SCF), C-kit, and inhibits PDGF- and SCF-mediated cellular events (Nadal & Olavarria 2004).

Arsenic trioxide (arsenic, Trisenox) has recently been recognized as an effective antineoplastic therapy, especially for the treatment of acute promyelocytic leukaemia (APL) (List et al 2003). Preclinical investigations indicate that the biological targets of arsenic extend to a variety of malignancies other than APL and include induction of apoptosis, non-terminal differentiation and suppression of proliferation and angiogenesis (List et al 2003). Arsenic apparently affects numerous intracellular signal transduction pathways and causes many alterations in cellular

function. It activates caspase-3 and mitogen-associated protein kinases (MAPKs), including p38, JNK and ERK, dose dependently. Moreover, arsenic induces apoptosis via reactive oxygen species (ROS), such as hydrogen peroxide, superoxide anion, hydroxyl radicals and nitric oxide (NO) (Miller et al 2002). In human CML cells that express Bcr-Ab1, arsenic treatment reduces Bcr-Ab1 levels and induces apoptosis (La Rosee et al 2002).

Favourable cytotoxicity and pro-apoptotic activity of imatinib in conjugation with arsenic and specific down-regulation of Bcr-Ab1 protein levels by arsenic in K562 cells indicate that arsenic in combination with imatinib might be useful for circumventing resistance to imatinib mono-therapy (Li et al 2002).

There have been several reports of serious life-threatening ventricular tachycardia, since arsenic prolongs the QT interval (Ohnishi et al 2000). In addition, torsades de pointes and sudden death have been reported with arsenic treatment (Barbey et al 2003; Drolet et al 2004). Also, PDGF has been shown to function importantly in the growth, development and function of most cardiovascular tissues. Ventricular myocytes respond to PDGF-BB with rapid mobilization of cell-associated Ca^{2+} and increased rates of protein synthesis, followed by marked increase in the rate of DNA synthesis. So, exposure to imatinib treatment may have an adverse impact on the heart of advanced phase CML patients (Brostrom et al 2002; Sohn et al 2003).

The aim of this work is to investigate the cardiotoxicity of arsenic/imatinib combination in a rat model, focusing on the cardiac peroxidative alterations and histopathological alterations that might develop due to this combination treatment.

Materials and Methods

Age-matched male Wister rats, 200 ± 20 g, were used. Throughout the investigations the rats were fed a standardized diet (Purina chow) and had free access to drinking water. The study adhered to the guidelines of the National Institutes of Health for the experimental use of animals. The institutional Ethical Committee for Research on Animals granted approval for the experiments.

Rats were divided into 4 groups. Control group received normal saline, a second control group was injected intraperitoneally with arsenic trioxide (BDH Chemicals Ltd, Poole, UK) 5 mg kg^{-1} daily for 10 days. Another group of rats received imatinib mesilate (Glivec, Novartis, Switzerland) orally at a dose of 30 mg kg^{-1} daily for 10 doses and a fourth group was treated with both drugs for 10 doses and all rats were sacrificed 48 h after the last dosing.

Rats were anaesthetized with ether and blood samples were taken by heart puncture. Hearts and livers were dissected out. A sample from each organ was fixed in 10% neutral formaldehyde for histopathological evaluation. Tissue samples were processed, embedded in paraffin, sectioned (3-μm thick), and stained with H&E. Another part from each rat's organ was washed with ice-cold saline, blotted with a piece of filter paper, weighed and homogenized (Biohomogenizer) in ice-cold bi-distilled water.

Serum creatinine and urea nitrogen levels were measured according to the methods of Bonsnes & Taussky (1945) and Hallet & Cook (1971), respectively. Serum creatine kinase-MB isoenzyme (CK-MB), lactate dehydrogenase (LDH), transaminases (AST & ALT) and bilirubin levels were estimated by using Randox Kits (Randox Laboratories Ltd, UK).

Tissue reduced glutathione (GSH), glutathione peroxidase (GPx) activity and total nitrate/nitrite (NOx) were determined with Ellman (1959), Paglia & Valentine (1967) and Miranda et al (2001) methods, respectively. Malondialdehyde (MDA) concentration in the tissue homogenates was determined according to the method of Ohkawa et al (1979).

The statistical significance of difference noted in the biochemical parameters was evaluated using one-way analysis of variance followed by Tukey HSD multiple comparisons test as a post-hoc.

Results

Serum creatine kinase-MB isoenzyme (CK-MB) in the control group was $7.1 \times 10^{-4} \pm 1.3 \times 10^{-4}$ IU (mg protein)$^{-1}$ (mean $\pm$ s.e.m.). Treatment of rats with arsenic or imatinib (or both) resulted in significant increases in the rate of change of serum CK-MB activity ($P < 0.01$). Simultaneous administration of both arsenic and imatinib to rats resulted in a non-additive significant increase in serum CK-MB activity compared with those rats treated with either arsenic or imatinib alone ($P < 0.01$) (Figure 1). Serum GPx activity

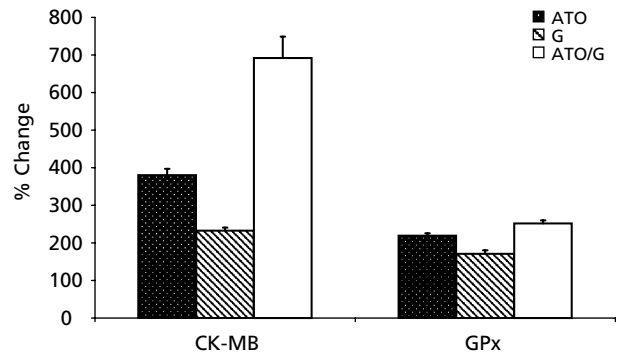

Figure 1 Effect of administration of arsenic trioxide (ATO), imatinib mesilate (G) or the two combined on serum creatine kinase isoenzyme (CK-MB) and glutathione peroxidase (GPx) in normal rats. The values shown are mean $\pm$ s.e.m. (analysis of variance followed by Tukey HSD as a post-hoc). The change rate was calculated as the percentage of change in each rat from the mean value of controls.

in the control group was $1.95 \times 10^{-2} \pm 3.33 \times 10^{-4}$ IU (mg protein)$^{-1}$. Administration of arsenic, imatinib, or the two combined to rats produced significant increases in the change rate of serum GPx activity compared with the control untreated rats ($P < 0.01$). Treatment with the combination of both arsenic and imatinib gave a non-additive increase in the serum GPx activity ($P < 0.01$) compared with the arsenic or imatinib-treated groups (Figure 1). Treatment with arsenic, imatinib, or both resulted in significant increases in serum LDH activity amounting to 92.5, 44 and 347% of those results obtained for the normal control rats, respectively. These treatments produced significant increases in serum AST activity ($P < 0.01$), but there was not a significant difference between the treated groups ($P > 0.05$). Serum bilirubin levels were significantly ($P < 0.01$) enhanced by arsenic-, imatinib-, or combination-treatment (185, 122 and 141% compared with the control untreated rats, respectively). Both serum creatinine and urea levels were significantly ($P < 0.01$) elevated by arsenic, imatinib, or combination treatment, although these values did not show a significant difference between the treated groups ($P > 0.05$).

Significant increases ($P < 0.01$) were observed in the change rate of heart reduced GSH content following treatment with arsenic, imatinib, or the two combined, compared with the control value ($2.9 \pm 0.065\,\mu$mol (g wet tissue)$^{-1}$). No significant difference was seen between rats treated with either arsenic alone or combined with imatinib ($P > 0.05$) (Figure 2, Table 1).

GPx activity level in the hearts of control rats was $7.18 \times 10^{-4} \pm 1.08 \times 10^{-5}$ IU (mg protein)$^{-1}$. Rats treated with arsenic alone or the arsenic/imatinib combination showed significant increases in GPx activity, although imatinib treatment resulted in significant reduction in the cardiac GPx activity level compared with the control group ($P < 0.01$). Moreover, treatment of rats with arsenic, imatinib, or the two combined revealed significant ($P < 0.01$) increases in heart MDA production compared with the control value (318.5 ± 6.27 nmol (g wet tissue)$^{-1}$). Combination arsenic/imatinib treatment produced a significant ($P < 0.01$) increase in heart MDA production level compared with groups treated with either arsenic or imatinib alone (Figure 2). The percentage change in total NOx was shown as a function of the mean value of the control saline treated rats ($299 \pm 6.7\,\mu$mol (g wet tissue)$^{-1}$) (Figure 2). Arsenic treatment significantly increased total NOx in the cardiac tissue by 36% of those of the control rats ($P < 0.01$). However, imatinib or arsenic/imatinib combination treatments produced significant ($P < 0.01$) reductions in the heart NOx content to about 67 and 65% of the mean control value, respectively (Figure 2).

The liver reduced GSH content increased significantly ($P < 0.01$) upon treatment with arsenic, imatinib, or the two combined, amounting to 89, 72.5 and 74% of those results obtained with saline treated control rats, respectively. GPx activity in the control rat's liver was $7.45 \times 10^{-4} \pm 2.03 \times 10^{-4}$ IU (mg protein)$^{-1}$. Treatment with arsenic, imatinib, or the two combined resulted in 34, −41 and 41% change ($P < 0.01$) from the control value, respectively. Moreover, the three treatments significantly increased liver MDA production level amounting to 25, 23 and 48% more than those results obtained with the normal control livers ($P < 0.01$, Table 1). However, those rats treated with arsenic alone or the arsenic/imatinib combination resulted in significant ($P < 0.01$) increases in liver total NOx, amounting to 17 and 49% compared with the control livers (Table 1).

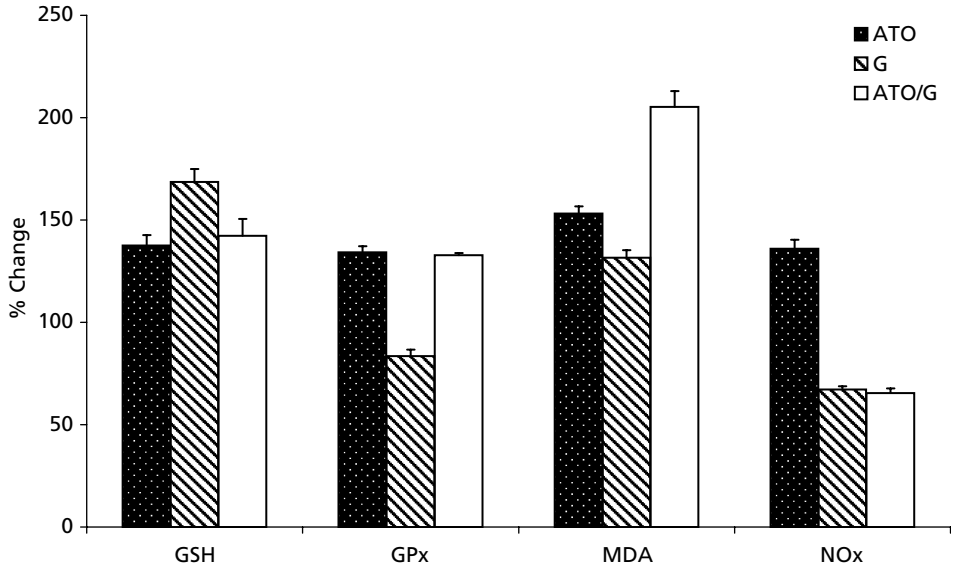

Figure 2 Peroxidative alterations (reduced glutathione (GSH), GPx activity and malondialdehyde (MDA) production levels) and total nitrate/nitrite (NOx) content induced by treatment with either arsenic trioxide (ATO) or imatinib mesilate (G) or combination of both in the cardiac tissue of normal rats. The values shown are mean ± s.e.m. (analysis of variance followed by Tukey HSD as a post-hoc). The change rate was calculated as the percentage of change in each rat from the mean value of controls.

Table 1 Serum biochemical parameters and peroxidative alterations of livers of rats treated with arsenic trioxide, imatinib mesilate, or both

Parameter	Group			
	Control	Arsenic	Imatinib	Arsenic/imatinib
LDH ($IU\,L^{-1}$)	134.80 ± 5.15^{a}	258.3 ± 11.5^{b}	193 ± 8.89^{ab}	599 ± 53
AST ($IU\,L^{-1}$)	144.3 ± 2.2	284.6 ± 9.0^{c}	286.00 ± 5.41^{c}	281.00 ± 5.02^{c}
ALT ($IU\,L^{-1}$)	47.0 ± 4.1^{d}	56.20 ± 2.28^{d}	60.8 ± 4.5^{a}	44.00 ± 2.88^{a}
Bilirubin ($mg\,dL^{-1}$)	0.68 ± 0.04	1.94 ± 0.09	1.51 ± 0.10^{e}	1.640 ± 0.066^{e}
Creatinine ($mg\,dL^{-1}$)	0.620 ± 0.057	1.310 ± 0.069^{f}	1.230 ± 0.058^{f}	0.970 ± 0.062
Urea ($mg\,dL^{-1}$)	33.70 ± 1.23	55.0 ± 4.8^{g}	49.80 ± 0.91^{g}	55.80 ± 2.21^{g}
Liver peroxidative alterations				
GSH (μmol (g wet tissue)$^{-1}$)	4.69 ± 0.18	8.87 ± 0.43^{a}	8.09 ± 0.62^{a}	8.17 ± 0.15^{a}
GPx (IU (mg protein)$^{-1}$)	$7.45 \times 10^{-4} \pm 2.03 \times 10^{-4}$	$9.98 \times 10^{-4} \pm 3.61 \times 10^{-4b}$	$4.38 \times 10^{-4} \pm 2.8 \times 10^{-4}$	$1.05 \times 10^{-3} \pm 6.68 \times 10^{-4b}$
MDA (nmol (g wet tissue)$^{-1}$)	277.00 ± 9.40	346.00 ± 11.78^{c}	340 ± 9^{c}	411.0 ± 9.8
Total NOx (μmol (g wet tissue)$^{-1}$)	358.00 ± 4.19^{d}	418.00 ± 8.85	340.50 ± 9.13^{d}	535.00 ± 21.28

Data are the mean ± s.e.m. of 6–10 rats. Means marked by the same superscript letters are not significantly different ($P > 0.05$, analysis of variance followed by Tukey HSD as a post-hoc).

Histopathological changes induced by arsenic or imatinib treatment revealed myocardial swelling, interstitial oedema and lymphocytic infiltration. Cardiac tissue of arsenic/imatinib-treated rats showed those changes induced by either arsenic or imatinib, in addition to fibroblastic proliferation, myocardial disorganization and myocardial necrosis (Figure 3, Table 2). No more than hydropic changes were seen in the livers of rats treated with arsenic, imatinib, or the two combined.

Discussion

The aim of the study was to define the cardiac safety of arsenic/imatinib combination in the rat model. Cardiac toxicity is frequently the indication for discontinuation of chemotherapeutic treatment in patients with tumours that remain sensitive to chemotherapy. Imatinib is fully approved for the treatment of patients with CML in chronic phase after failure of interferon-α therapy in blast crisis and in accelerated phase (Cohen et al 2005). This work revealed that imatinib treatment produced significant increases in serum CK-MB, GPx, LDH and AST activity compared with control untreated rats. Moreover, imatinib-treated rats showed significant reductions in the cardiac GPx activity and total NOx concentration, as well as elevations in reduced-GSH content and MDA production.

A cross talk between cardiac myocytes and non-myocytes via humoral factors plays an important role in the development of cardiac growth. Exploration of the protective effect of humoral factors produced from non-myocytes against acute myocardial injury might lead to mechanistic insights. Thus, inhibition of PDGF receptors by imatinib might contribute to inhibition of neovascularization and collaterals formation leading to hypoxia and oxidative damage (Armstrong et al 1998; Li et al 2004; Nilsson et al 2004). c-Ab1 has been implicated in cell response to DNA damage and oxidative stress. Imatinib-induced inhibition of c-Ab1 might contribute to endogenous antioxidant depletion with activation of cellular Nrf2 expression level (Jaiswal 2004). Nrf2 binds to antioxidant response element (ARE) in the nucleus. This is presumably to keep the expression of antioxidant enzymes in check to maintain the cellular defences active, or to rapidly restore induced enzymes to normal levels (Yin et al 1998). Thus, c-Ab1 might be important in the control of cellular antioxidant defence mechanisms. Inhibition of c-Ab1 in the cardiac myocytes by imatinib treatment might contribute to attenuation of cardiac GPx activity. Reduction of GPx activity due to imatinib treatment was associated with significant increase in cardiac GSH content, which might be attributed to GSH rebound through enhancement of γ-glutamylcysteine synthase gene expression (Yeh et al 2002). Moreover, treatment of rats with imatinib resulted in significant increase in cardiac MDA production level due to imatinib-induced myocardial c-Ab1 and PDGF receptor inhibition, contributing to peroxidative-induced myocardial damage (Jaiswal 2004; Li et al 2004; Nilsson et al 2004).

Rats treated with arsenic showed significant increases in cardiac GSH content, GPx activity, MDA production

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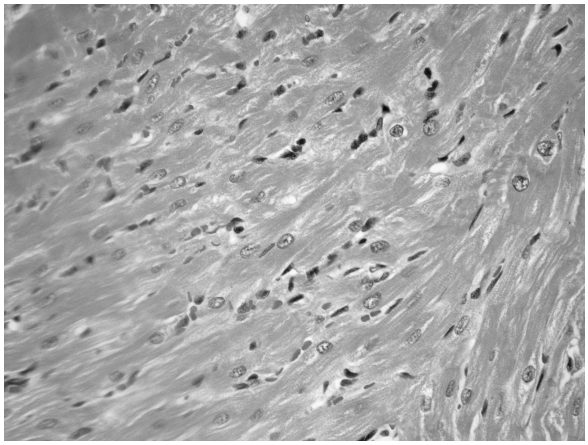

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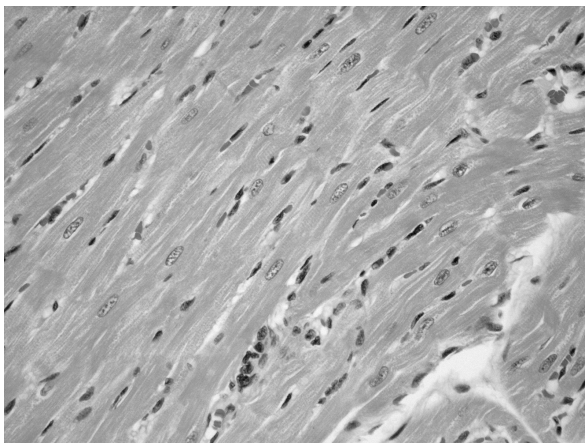

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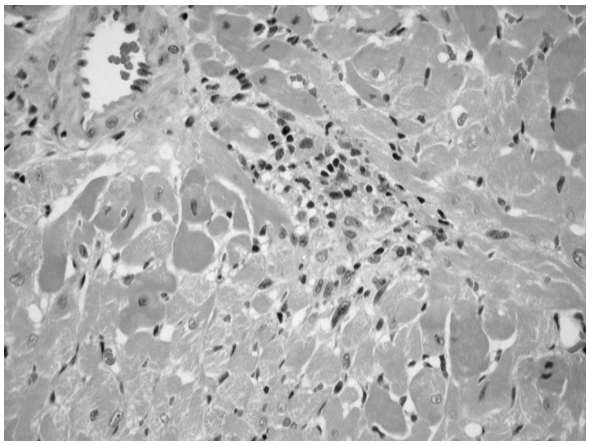

Figure 3 Photomicrograph of myocardial section taken from a rat treated with imatinib mesilate (A) or arsenic trioxide (B) depicting myocardial swelling, interstitial oedema and lymphocytic infiltration (H&E × 400) and photomicrograph of myocardial section taken from a rat treated with arsenic/imatinib combination (C) revealing fibroblastic proliferation, myocardial disorganization and myocardial necrosis (H&E × 400).

Table 2 Myocardial histopathological changes induced by treatment of normal rats with arsenic trioxide or imatinib mesilate, or both

Group	Median histopathological changes (Score, +)
Control	0
Arsenic	2 (2–2)[a]
Imatinib	2 (2–2)[a]
Arsenic/imatinib	3.5 (3–4)

Values represent the median with the range in parenthesis (n = 6–10). Histopathological scores were graded from 0 to 4: 0 represents no histopathological changes; 1+ for myocardial swelling and interstitial oedema; 1+ for fibroblastic proliferation and myocardial disorganization; 1+ for myocardial necrosis and 1+ for myocardial lymphocytic infiltration. Medians marked by the same superscript letters are not significantly different ($P > 0.05$, Kruskal–Wallis analysis of variance).

and total NOx concentration, as well as serum CK-MB and GPx activity. These results are in accordance with those reported by Yeh et al (2002). Treatment with arsenic induces oxidative stress via ROS generation, depletion of cellular endogenous antioxidant reserve, release of cytochrome C and apoptosis-inducing factor (AIF) from the mitochondria, activation of caspases, down-regulation of Bcl-2 and Bcl-x(L) and up-regulation of Bax expression leading to cellular apoptosis (Gupta et al 2003). Moreover, arsenic compounds may trigger apoptosis, micronuclei formation and DNA strand breaks by increasing cellular nitric oxide (NO) and superoxide levels (Gurr et al 1999). The increase in myocardial total NOx concentration following arsenic treatment might reflect an increase in inducible NO that might inhibit DNA repair (Bau et al 2001). Also, pyruvate dehydrogenase (PDH) is an enzyme that is supersensitive to arsenic. Inhibition of PDH blocks the aerobic oxidation of glucose and inhibits the oxidative phosphorylation of ADP leading to cellular adenine nucleotide degradation (Vlessis et al 1991; Borutaite & Brown 2003).

Treatment of rats with the arsenic/imatinib combination resulted in significant increases in serum CK-MB, GPx, LDH and AST activity, as well as serum bilirubin, creatinine and urea levels. Moreover, the arsenic/imatinib combination produced significant increases in cardiac GSH content, GPx activity and MDA production, in addition to a reduction in total NOx content. Augmentation of cardiotoxicity following treatment with the arsenic/imatinib combination, compared with arsenic- or imatinib-treated groups, might be attributed to imatinib-induced PDGF receptor and c-Ab1 blockade in addition to arsenic-induced oxidative stress as previously discussed.

Imatinib mesilate is a substrate as well as a modulator of human P-gp, suggesting that imatinib–drug interactions may occur via P-gp (Hamada et al 2003). Moreover, arsenic induces multi-drug resistance (MDR)-associated proteins that might contribute to arsenic efflux; thus blockade of MDR-associated protein increases cellular accumulation of arsenic (Vernhet et al 2003). So, it might be speculated that imatinib modulates cardiac

arsenic accumulation, exacerbating myocardial oxidative stress and reduced total NOx concentration in the cardiac tissue due to inhibition of PDGF, as well as cellular Akt that might be of great importance in the activation of endothelial nitric oxide synthase (eNOS) (Takahashi & Mendelsohn 2003), explaining the imatinib-induced total NOx content depletion. Therefore, Akt inhibition induced by imatinib treatment might contribute to partial reduction in cardiotrophin-1 (CT-1)-mediated NF-κB activation, as well as the cytoprotective effects of CT-1 against hypoxia stress induced by the co-administration of arsenic (Craig et al 2001). Also, imatinib-induced blockade of PDGF (physiological PKC-delta isoenzyme activator) might contribute to an increase in the degradation of neutral ceramidase and cardiac ceramide accumulation (Franzen et al 2002). In addition, imatinib induces expression of COX-2 in a dose-dependent manner with concomitant accumulation of prostaglandin E2 (Johnson et al 2004). Increased COX-2 expression might contribute to play a role in oxidative alterations in the heart.

Comparable results were obtained with the rat liver following treatment with arsenic, imatinib or both. However, there are preferential exacerbation of toxic effects for both drugs either singly or in combination to the heart. Histopathological investigation of the rat liver showed only hydropic changes in all treated groups. This might be attributed to the increase in NO production (reflected in the total NOx content level) compared with the heart especially following arsenic/imatinib combination, since NO has been reported to prevent the increase as well as directly inhibit caspase-3-like activity in hepatocytes (Kim et al 1997).

The dose of arsenic trioxide was 5 mg kg^{-1} daily, which has been shown to produce plasma concentration of arsenic within the range of those presented in arsenic-treated APL patients (Li et al 2002). In this study, cardiotoxicity developed following short-term treatment, especially when co-administered with imatinib. In-vivo distribution and biotransformation in addition to the health condition of cancer patients might be completely different compared with the rat model. These results might warrant orientation to explore the cardiotoxicity of either imatinib or the arsenic/imatinib combination in cancer patients in parallel with studies regarding their anti-tumour activity.

In conclusion, both arsenic and imatinib might possess significant cardiotoxity and cardiac function should be monitored during treatment with either arsenic, imatinib, or a combination of the two, especially in the presence of pre-existing cardiac dysfunction. Also, pharmacokinetic and pharmacodynamic studies are warranted, especially when both drugs are used in combination with other chemotherapeutic drugs known to have cardiotoxic side effects.

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