Cardiology in the Young (2016), **26**, 431–438
doi:10.1017/S1047951115000360

Original Article

The role of curcumin as an inhibitor of oxidative stress caused by ischaemia re-perfusion injury in tetralogy of Fallot patients undergoing corrective surgery

Rubiana Sukardi,[1] Sudigdo Sastroasmoro,[2] Nurjati C. Siregar,[3] Mulyadi M. Djer,[2] Fransciscus D. Suyatna,[4] Mohammad Sadikin,[5] Nurhadi Ibrahim,[6] Sri E. Rahayuningsih,[7] Arief B. Witarto[8]

[1]*Integrated Cardiovascular Service, Dr Cipto Mangunkusumo General Hospital;* [2]*Department of Child Health, Cardiology Division;* [3]*Department of Pathology Anatomy;* [4]*Department of Pharmacology;* [5]*Department of Biochemistry;* [6]*Department of Physiology, Medical Faculty, University of Indonesia, Jakarta;* [7]*Department of Child Health, Cardiology Division, Medical Faculty, University of Padjajaran, Bandung;* [8]*Research and Development Laboratory, Dharmais Hospital National Cancer Center, Jakarta, Indonesia*

Abstract *Background:* Cardiopulmonary bypass during tetralogy of Fallot corrective surgery is associated with oxidative stress, and contributes to peri-operative problems. Curcumin has been known as a potent scavenger of reactive oxygen species, which enhances the activity of antioxidants and suppresses phosphorylation of transcription factors involved in inflamation and apoptosis. *Objectives:* To evaluate the effects of curcumin as an antioxidant by evaluating the concentrations of malondialdehyde and glutathione, activity of nuclear factor-kappa B, c-Jun N-terminal kinase, caspase-3, and post-operative clinical outcomes. *Methods:* Tetralogy of Fallot patients for corrective surgery were randomised to receive curcumin (45 mg/day) or placebo orally for 14 days before surgery. Malondialdehyde and glutathione concentrations were evaluated during the pre-ischaemia, ischaemia, re-perfusion phases, and 6 hours after aortic clamping-off. Nuclear factor-kappa B, c-Jun N-terminal kinase, and caspase-3, taken from the infundibulum, were assessed during the pre-ischaemia, ischaemia, and re-perfusion phases. Haemodynamic parameters were monitored until day 5 after surgery. *Results:* In all the observation phases, malondialdehyde and glutathione concentrations were similar between groups. There was no significant difference in nuclear factor-kappa B activity between the groups for three observations; however, in the curcumin group, c-Jun N-terminal kinase significantly decreased from the pre-ischaemia to the re-perfusion phases, and caspase-3 expression was lower in the ischaemia phase. Patients in the curcumin group had lower temperature and better ventricular functions, but no significant differences were found in mechanical ventilation day or length of hospital stay in the two groups. *Conclusion:* Cardioprotective effects of curcumin may include inhibition of the c-Jun N-terminal kinase pathway and caspase-3 in cardiomyocytes, particularly in the ischaemia phase.

Keywords: Curcumin; ischaemia re-perfusion injury; apoptosis

Received: 11 August 2014; Accepted: 15 February 2015; First published online: 28 April 2015

Tetralogy of Fallot occurs in three of every 10,000 live births and is the most common cause of cyanotic CHD,[1] which requires open heart corrective surgery. Patients who undergo tetralogy of Fallot repair are at high risk for ischaemia re-perfusion injury caused by the use of the cardiopulmonary bypass machine and sudden blood restoration after a period of aortic clamping-off.[2] Our study in 2013 showed that 22% of patients with tetralogy of Fallot who underwent total correction

Correspondence to: R. Sukardi, Cipto Mangunkusumo Hospital, Integrated Cardiovascular Center, Jalan Diponegoro 71, Jakarta 10430, Indonesia. Tel: +6 221 390 5839; E-mail: rubiana_sukardi@yahoo.com

showed an increase in procalcitonin levels (the mean procalcitonin value was 89.55 ng/ml) and clinical signs of systemic inflammatory response syndrome 24 hours after the procedure.[3] It has been known that during restoration of blood flow to the ischaemic heart an extra burst of free radicals occurs. Free radicals cause lipid peroxidation, and lipid peroxidation end-products, malondialdehyde, have been used to assess oxygen free radical-mediated injury in re-perfusion injury.[4] Re-perfusion injury also leads to the depletion of glutathione, the major intracellular antioxidant, which is important for the maintenance of cellular proteins and lipids in their functional phases. Pro-inflammatory cytokines are activated after cardiopulmonary bypass. Nuclear factor-kappa B is involved in regulating inflammatory signal transduction. Furthermore, myocardial ischaemia and ischaemia/re-perfusion may also activate several protein kinase families, including c-Jun N-terminal kinase, the primary kinase involved in myocardial ischaemic apoptosis.[5]

Curcumin, an active component of turmeric, is a well-known antioxidant. It scavenges reactive oxygen species and has anti-inflammatory properties. These properties appear to be mediated by the inhibition of cytokine production and the activation of transcription factors such as nuclear factor-kappa B and several kinases upstream of c-Jun N-terminal kinase.[6] We assessed the effect of curcumin on decreasing myocardial ischaemia re-perfusion injury during cardiopulmonary bypass and by attenuating the appearance of apoptosis caspase-3 of cardiomyocytes. In addition, we also measured the post-operative clinical outcomes.

Methods

Design

This single-centre, double-blind, randomised placebo-controlled trial was performed to evaluate the effects of curcumin on malondialdehyde and glutathione levels, nuclear factor-kappa B, and c-Jun N-terminal kinase transcription factor activity, as well as on caspase-3 expression, in children aged between 1 and 6 years, who underwent tetralogy of Fallot corrective surgery. This study was approved by the Ethics Research Committee of the University of Indonesia Medical School/Cipto Mangunkusumo Hospital, Jakarta. Written informed consent was obtained from parents. Randomisation was carried out by block permutation. Patients in the placebo group received *saccharum lactis*, whereas those in the curcumin group received 45 mg oral curcumin daily (containing 98% curcuminoid), both for 14 days before surgery. This dose was considered to be safe and effective based on the study by Alwi, which showed 45 mg of curcumin for 1 month can suppress inflammatory response in the coronary acute syndrome.[7] The team chose 14 days of treatment for safety reasons, as this is the first study on curcumin to be carried out in children with prolonged hypoxia.

Study criteria

We included patients undergoing elective tetralogy of Fallot repair, and we excluded patients with other major anomalies, McGoon ratio <1.5, patients with previous palliative procedures, or pulmonary atresia with ventricular septal defect. Pre-operative routine blood investigation, liver and renal functions, as well as coagulation profiles were taken before and after drug administration in both groups.

Measurements

Blood specimens for malondialdehyde and glutathione measurements were collected from mixed vein blood (right atrium) for the pre-ischaemia phase and 6 hours after surgery. Specimens for the ischaemia and re-perfusion phases were taken from the coronary sinus. Serum malondialdehyde levels were evaluated by spectrophotometry based on the reaction between malondialdehyde and thiobarbituric acid, as described by Wills[8] and Devasagayam et al.[9] Serum glutathione was evaluated by glutathione assay, as described by Ellman;[10] measurements using this method require total protein concentration, which was determined as described by Peterson.[11]

Cardiomyocyte tissue biopsy sample was taken directly from the infundibulum. These specimens were used for nuclear factor-kappa B and c-Jun N-terminal kinase enzyme-linked immunosorbent assay analysis and for the measurement of caspase-3 expression. Phosphorylated nuclear factor-kappa B and c-Jun N-terminal kinase were analysed from nuclear proteins of the cardiomyocyte tissue biopsy. In order to extract these proteins, we used a Nuclear Extract Kit (Active Motif, Carlsbad, California, United States of America). For enzyme-linked immunosorbent assay analysis, phosphorylated nuclear factor-kappa B was measured using a TransAM NFκB p50 transcription factor assay kit, and for phosphorylated c-Jun N-terminal kinase we used a Face JNK ELISA Kit (Active Motif). These enzyme-linked immunosorbent assay analyses yielded absorbance values for active nuclear factor-kappa B and c-Jun N-terminal kinase proteins, but not the absolute values of these transcription factors. As phosphoproteins are characteristically unstable, it was difficult to prepare a standard phosphoprotein of fixed concentration. To maintain the quality of

examinations, all specimens were examined under similar conditions, from the preparation to nuclear protein extraction phases. We also avoided freezing and thawing nuclear protein extracts.

The expression of caspase-3 was measured using a specific, cleaved caspase-3 antibody (Asp175) (Cell Signaling Technology, Beverly, Massachusetts, United States of America). Primary antibodies were stained with the cleaved caspase-3 antibody (diluted 1:2000) and visualised using a Star Trek Universal HRP Detection System Kit (Biocare Medical, Concord, California, United States of America) according to the manufacturer's instructions.

Primary outcomes

The primary outcomes were serial serum malondialdehyde and glutathione levels taken at the pre-ischaemia, ischaemia, and re-perfusion phases, as well as at 6 hours after aortic clamping-off. Other primary outcomes were serial measurements of active nuclear factor-kappa B and c-Jun N-terminal kinase proteins, as well as caspase-3 expression, as obtained from the right ventricular outflow tract endocardial (infundibular) biopsy during the pre-ischaemia, ischaemia, and re-perfusion phases.

Secondary outcomes

The secondary outcomes were the haemodynamic parameters such as body temperature, lactate levels, oxygen saturation, mixed vein saturation, inotropic score, urine output, creatinine level, cardiac index, and systemic vascular resistance index taken at 6, 24, and 48 hours after ICU admission, as well as on the 5th day after surgery. Time to extubation and length of stay in the ICU and hospital were also recorded.

Statistical analysis

Statistical analysis was performed with SPSS 20.0 software. We used the independent t-test for normally distributed numerical data; otherwise, we used the Mann–Whitney test. A general linear model test was used for variables measured more than once. A p value <0.05 was considered to be statistically significant.

Results

Study patients

This study was carried out from July, 2012 to July, 2013. Of the 50 recruited patients, five were excluded because of incomplete data. The age range of the patients was from 46 months to 6 years and 11 months. There were 22 patients in the curcumin group and 23 in the placebo group. Some patients did not have surgery immediately following the 14 days of treatment due to illness or lack of ICU beds. Both groups, however, had similar means of treatment times – that is, 20 days. They also had similar tetralogy of Fallot anatomical indicators for corrective surgery (Nakata index and McGoon ratio), aortic clamping duration, and cardiac bypass duration. Characteristics data of the patients are shown in Table 1.

We examined complete blood counts, liver and kidney functions, and coagulation profiles before and 14 days after treatment to assess the effects

Table 1. Characteristics of the patients (n = 45).

	Groups	
Variable	**Placebo (n = 23)**	**Curcumin (n = 22)**
Age (months) (mean (SD))	46.6 (20.2)	49.4 (23.8)
Body weight (kg) (mean (SD))	12.2 (3.1)	12.1 (2.9)
Height (cm) (mean (SD))	91 (12)	90.7 (10.9)
BSA (m^2) (mean (SD))	0.54 (0.10)	0.56 (0.10)
Duration of treatment (days) (mean (SD))	20 (9.3)	20.1 (6.7)
PA half size (mm) (mean (SD))	8.2 (1.1)	7.8 (0.9)
McGoon ratio (mean (SD))	1.9 (0.5)	1.9 (0.4)
Nakata index (mean (min–max))*	254 (223.5–291.2)	259.6 (227–306.5)
TAPSE (mm) (mean (SD))	18.59 (1.87)	19.33 (2.17)
EF (%) (mean (SD))	70.14 (6.95)	69.44 (6.15)
FS (%) (mean (SD))	36.45 (6.07)	38.22 (9.69)
Ao clamping duration (minute) (mean (min–max))*	31.5 (28.4–35.3)	30.3 (27.4–33.9)
Cardiac bypass duration (minute) (mean (min–max))*	79.6 (73.8–86.4)	82.1 (75.1–90.4)

Ao = aortic; BSA = body surface area; CI = confidence interval; EF = ejection fraction; FS = fractional shortening; max = maximal; min = minimal; PA half size = pulmonary artery half size; TAPSE = tricuspid annular plane systolic excursion

*t-test after data transformation, presented as geometrical means (95%CI, minimal–maximal)

of curcumin. There were no significant differences between laboratory results before and after the treatment (data not shown).

Primary outcomes

Malondialdehyde and glutathione serum concentrations. Both groups showed increased malondialdehyde concentrations at the ischaemia and re-perfusion phases; however, the geometric means were not significantly different between groups among the four observation phases. Glutathione concentrations in the four observation phases were also not significantly different between groups. The glutathione concentration increased in the ischaemia and re-perfusion phases of both the groups, and then decreased to baseline levels in the 6 hours after aortic clamping-off phase (Table 2).

Nuclear factor-kappa B and c-Jun N-terminal kinase protein activities in the nuclei of cardiomyocytes. The median values of nuclear factor-kappa B of the curcumin group in the pre-ischaemia and ischaemia phases were higher than that of the placebo group; however, median values were similar between groups in the re-perfusion phase. Mean delta nuclear factor-kappa B differences in all observation phases were not significantly different (delta nuclear factor-kappa B data not shown). The median active c-Jun N-terminal kinase protein was similar in both the groups; however, the mean delta c-Jun N-terminal kinase was significantly different in the two groups, from the pre-ischaemia to the re-perfusion phases –0.184 decrease in the curcumin group and 0.080 in the placebo group (p = 0.048) (Table 3).

Caspase-3 expression in cardiomyocytes. The percentage of caspase-3 expression was measured from the mean number of cells with caspase-3 expression divided by the total number of cardiomyocytes observed in a specimen of myocardial tissue. The examination of caspase-3 with immunohistochemistry showed

Table 2. Mean MDA and GSH serum concentrations in the 4 phases of observation.

	Groups		
MDA/GSH (nmol/mg protein)	**Placebo (n = 23)**	**Curcumin (n = 22)**	**p**
MDA1 (mean) (min–max)	0.017 (0.015–0.020)	0.017 (0.014–0.022)	0.938
MDA2 (mean) (min–max)	0.045 (0.038–0.053)	0.040 (0.032–0.049)	0.355
MDA3 (mean) (min–max)	0.045 (0.038–0.055)	0.041 (0.033–0.052)	0.556
MDA4 (mean) (min–max)	0.026 (0.021–0.031)	0.028 (0.021–0.036)	0.658
GSH1 (mean) (min–max)	0.83 (0.68–1.01)	0.87 (0.72–1.05)	0.758
GSH2 (mean) (min–max)	2.63 (2.16–3.19)	2.43 (2.08–2.85)	0.549
GSH3 (mean) (min–max)	2.59 (2.12–3.15)	2.36 (2.00–2.78)	0.488
GSH4 (mean) (min–max)	0.95 (0.80–1.11)	0.91 (0.79–1.06)	0.765

MDA = malondialdehyde; GSH = glutathione; min = minimal; max = maximal; CI = confidence interval
Analysis with GLM after data transformation, mean of 95%CI. MDA 1/GSH 1: MDA/GSH in pre-ischaemia phase; MDA 2/GSH 2: MDA/GSH in ischaemia phase; MDA 3/GSH 3: MDA/GSH in re-perfusion phase; MDA 4/GSH 4: MDA/GSH in 6 hours after aortic clamping-off (in the ICU)

Table 3. Active NFκB and JNK protein in cardiomyocyte nuclei in the three phases of observation.

	Groups		
NFκB/JNK	**Placebo (n = 23)**	**Curcumin (n = 22)**	**p**
NFκB 1 (median) (P25–P75)	0.536 (0.472–0.917)	0.626 (0.535–0.750)	0.648*
NFκB 2 (median) (P25–P75)	0.525 (0.454–0.699)	0.646 (0.487–1.008)	0.235*
NFκB 3 (median) (P25–P75)	0.556 (0.386–0.660)	0.574 (0.485–0.733)	0.273*
JNK 1 (median) (P25–P75)	0.624 (0.559–0.693)	0.761 (0.577–0.841)	0.134*
JNK 2 (median) (P25–P75)	0.534 (0.501–0.722)	0.592 (0.452–0.655)	0.961*
JNK 3 (median) (P25–P75)	0.511 (0.459–0.598)	0.544 (0.427–0.618)	0.883*
Delta JNK 1–2 (mean (SD))	0.051 (0.219)	0.125 (0.242)	0.354**
Delta JNK 2–3 (mean (SD))	0.029 (0.268)	0.059 (0.194)	0.705**
Delta JNK 1–3 (mean (SD))	0.080 (0.110)	0.184 (0.182)	**0.048****

NFκB = nuclear factor-kappa B; JNK = c-Jun N-terminal kinase P25; P75 = 25th percentile and 75th percentile
NFκB 1/JNK 1: NFκB/JNK in the nucleus of cardiomyocytes at the pre-ischaemia phase; NFκB 2/JNK 2: NFκB/JNK in the nucleus of cardiomyocytes at the ischaemia phase; NFκB 3/JNK 3: NFκB/JNK in the nucleus of cardiomyocytes at the re-perfusion phase
*Mann–Whitney test
**Unpaired t-test

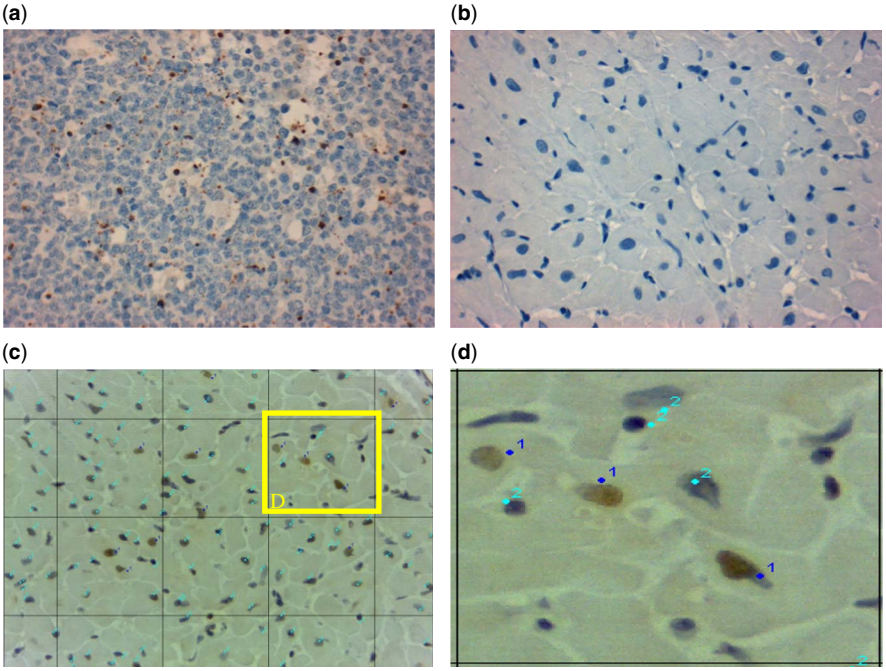

Figure 1.
*Expression of caspase-3 by immunohistochemistry (original magnification 400 ×). The brown staining in cardiomyocytes indicates the presence of caspase-3, and the blue staining indicates the absence of caspase-3. (**a**) Positive control reaction in human tonsils, (**b**) negative control reaction in the myocardium, (**c**) caspase-3 expression during the ischaemia phase in the myocardium, (**d**) magnified area with cell colour difference as a result of reaction with caspase-3 antibody. Numbers 1 and 2 are nuclei with and without caspase-3 expression, respectively.*

Table 4. Caspase-3 in cardiomyocytes in the three observation phases.

	Groups		
Observation phase	Placebo (n = 23)	Curcumin (n = 22)	p
Pre-ischaemia (%) (median (1st–3rd quartile))	2 (0–6.25)	1 (0–11.5)	0.613
Ischaemia (%) (median (1st–3rd quartile))	2 (1–5)	1 (0–1.5)	**0.035**
Re-perfusion (%) (median (1st–3rd quartile))	1 (0–5.5)	1 (0–2.5)	0.432

Mann–Whitney test
Caspase-3 expression is presented in 10^{-3}
Data are presented as median (1st–3rd quartile)

optimal result. Immunohistochemical analysis of caspase-3 yielded the following: cardiomyocyte nuclei with caspase-3 expression were stained brown, whereas nuclei without caspase-3 expression were stained blue (Fig 1). Caspase-3 expression in the ischaemia phase was significantly lower in the curcumin group than in the placebo group (p = 0.035). There was no significant difference between the pre-ischaemia and re-perfusion phases between groups (Table 4).

Secondary outcomes

Post-surgery haemodynamics. The mean body temperatures in the curcumin group were significantly lower compared with the placebo group in the three observation phases (6, 24, and 48 hours after ICU admission). Mean arterial blood oxygen saturation at 6 hours after aortic clamping-off in the curcumin group was significantly higher, although the difference was not clinically significant. The haemodynamic data are shown in Table 5.

Dopamine and milrinone were administered as a continuous intravenous drip. Inotropic scores were not significantly different between the two groups. Serial echocardiographic examinations for right and left ventricular functions were performed before surgery until 5 days after surgery. Ventricular functions were similar between groups before surgery (data not shown). Right ventricular function – tricuspid annular plane systolic excursion – decreased after

Table 5. Post-surgery haemodynamic data.

Indicator/observation phase	Groups		p
	Placebo (n = 23)	Curcumin (n = 22)	
Temperature (°C)			
I (mean) (SD)	37.5 (0.62)	37.0 (0.66)	**0.023***
II (mean) (SD)	37.5 (0.77)	37.1 (0.60)	**0.036***
III (mean) (SD)	37.3 (0.80)	36.9 (0.58)	**0.043***
SpO_2 (%)			
I (mean) (SD)	98.8 (1.30)	99.3 (0.88)	**0.041***
II (mean) (SD)	98.6 (1.90)	98.5 (1.74)	0.628*
III (mean) (SD)	98.6 (1.61)	97.4 (4.04)	0.542*
Inotropic score			
I (mean) (min–max)	11.4 (9.34–13.83)	9.9 (8.48–11.56)	0.288**
II (mean) (min–max)	11.6 (8.74–15.49)	8.5 (6.35–11.42)	0.144**
III (mean) (min–max)	9.3 (6.77–12.66)	10.1 (5.82–17.51)	0.744**
Right ventricle (TAPSE) (mm)			
I (mean) (SD)	8.58 (2.47)	11.23 (3.97)	**0.012***
II (mean) (SD)	10.02 (2.88)	13.44 (4.10)	**0.003***
III (mean) (SD)	10.03 (2.92)	14.53 (3.76)	**<0.001***
IV (mean) (SD)	12.91 (4.16)	16.73 (2.78)	**0.001***
Left ventricle (EF) (%)			
I (mean) (SD)	59.75 (9.17)	66.51 (7.43)	**0.013***
II (mean) (SD)	59.99 (7.51)	67.57 (7.50)	**0.002***
III (mean) (SD)	61.33 (5.55)	71.38 (6.16)	**<0.001***
IV (mean) (SD)	63.90 (7.73)	71.54 (5.53)	**0.001***
Left ventricle (FS) (%)			
I (mean) (SD)	30.31 (6.98)	34.55 (5.30)	**0.034***
II (mean) (SD)	29.46 (5.43)	32.48 (6.38)	**0.001***
III (mean) (SD)	30.59 (3.82)	38.08 (5.73)	**<0.001***
IV (mean) (SD)	32.75 (5.56)	39.02 (5.14)	**0.001***

I = examination 6 hours after aortic clamping-off (in ICU); II = examination 24 hours after surgery; III = examination 48 hours after surgery; IV = examination 5 days after surgery; CI = confidence interval; EF = ejection fraction; FS = fractional shortening; GLM = general linear model; SpO_2 = arterial oxygen saturation; TAPSE = tricuspid annular plane systolic excursion

*Analysis with GLM

**Analysis with GLM after data transformation. Data are presented as geometric means (95%CI)

surgery in both the groups; however, the curcumin group had significantly better tricuspid annular plane systolic excursion. The tricuspid annular plane systolic excursion in both the groups remained under baseline values at 5 days after surgery. The systolic left ventricular function – ejection fraction and fractional shortening – in the placebo group decreased from baseline values until 5 days after surgery, although the mean values were in the normal range. The mean ejection fraction and fractional shortening differed significantly between the two groups at 6, 24, and 48 hours as well as at 5 days after surgery.

Discussion

To the best of our knowledge, this is the first study to use curcumin treatment in an effort to reduce the injurious effect of ischaemia and re-perfusion in children with tetralogy of Fallot undergoing corrective surgery. The threshold of tolerance to curcumin is high, according to a study of titrated doses given to children with ulcerative colitis aged between 8 and 18 years.[12] During the pre-surgery observation, no clinical symptoms were caused by the treatment and all the patients received their complete doses. The dosage used in this study was 45 mg of curcumin/day for 14 days before surgery, the lowest dose ever given to adults.[13]

A study in children with congenital heart defects found that their malondialdehyde levels were higher compared with the normal population; furthermore, their total antioxidant capacity was significantly decreased.[14] In cyanotic patients who underwent corrective surgery, malondialdehyde levels further increased and glutathione levels depleted.[15,16–19]

In a rat study, curcumin treatment with a dosage of 100 mg/kg intra-peritoneally 1 hour before re-perfusion significantly increased antioxidant activity and lowered malondialdehyde concentrations.[20] Other animal studies using intravenous curcumin doses of 20 mg/kg body weight or 40 mg/kg before the ischaemia episode also reported lower malondialdehyde

concentrations.[21] Curcumin was found to decrease lipid peroxidation by maintaining antioxidant activity.[16] Our differing results may have been caused by our low dose of curcumin, the timing of curcumin administration before the re-perfusion phase, or the difference in malondialdehyde concentrations, because the ischaemia re-perfusion injury did not disrupt cardiomyocyte cell membranes. As such, sub-cellular malondialdehyde and its precursors were not released, and therefore were undetected by the assay.[22]

Ischaemia re-perfusion myocardial injury activates the transduction pathways of nuclear factor-kappa B and c-Jun N-terminal kinase phosphorylation,[23–25] and can cause apoptosis or cellular necrosis.[26,27] A study in mouse hearts found that c-Jun N-terminal kinase phosphorylation doubled after an ischaemic period of 20 and 15 minutes after re-perfusion.[28] Another study showed elevated c-Jun N-terminal kinase activation during the ischaemia and re-perfusion phases,[29] whereas a study by Shao et al[30] found that increased c-Jun N-terminal kinase activation only occurred during re-perfusion. A study in animal cardiac cells with 10 μM curcumin, administered before the ischaemia phase, showed it to be the most effective time for lowering nuclear factor-kappa B and c-Jun N-terminal kinase expressions, as well as for lowering cell death.[31]

In our study, expression of active nuclear factor-kappa B in every phase of observation was similar between groups, perhaps due to the low dose of curcumin; however, the effects of curcumin on c-Jun N-terminal kinase suppression and caspase-3 expression were seen from the pre-ischaemia to the re-perfusion phases and the ischaemia phase, respectively.

The curcumin group had significantly lower temperature than the placebo group. This may be caused by the effect of curcumin in inhibiting cytokine production pathways such as tumor necrosis factor α, interleukin-1β, and interleukin-8 from inflamed cells.[32,33] Increased temperature in children after heart surgery is usually a response to systemic inflammation caused by various processes including blood contact with a cardiac pulmonary bypass machine,[34,35] ischaemia re-perfusion injury secondary to aortic clamping-off, and endotoxaemia.

Inotropic scores are widely used for early post-surgical result parameters. Maximal inotropic score is a superior indicator compared with low cardiac output for predicting early post-heart surgery results.[36] Arterial oxygen saturation may also provide haemodynamic status information.[37] In our study, curcumin did not appear to affect inotropic scores in our patients.

During ischaemia re-perfusion, a heart may undergo changes such as depressed contraction function, arrhythmia, and gene expression.[38] A few studies reported that curcumin protected heart performance from ischaemia re-perfusion injury.[31,39] A curcumin dose of 150 mg/kg orally at the re-perfusion phase in animals, with a 45-minute ischaemia period, repaired ventricular function by lowering malondialdehyde levels, protecting the extracellular matrix from degradation by lowering the activity of matrix metalloproteinase, and inhibiting fibroblast accumulation caused by expression of α-smooth muscle actin.[40]

The left and right ventricular contractility functions were better in the curcumin group than in the placebo group, which may have been due to a curcumin effect on apoptosis suppression.

Study limitations and strengths

A limitation of our study was that we did not obtain data for malondialdehyde and glutathione levels before treatment with curcumin or placebo, due to the difficulty of obtaining blood specimens from children susceptible to severe hypoxaemic shock. As such, we used the pre-ischaemia data as the "initial" assessment. Another limitation was the use of a single curcumin dosage for all patients, who had a wide range of body weights.

Nevertheless, the advantages of this study were that the specimens for oxidative stress levels were collected from the right atrium and coronary sinus, and the tissue specimens for transcription factor examinations were taken from the myocardium, reflecting actual myocardial conditions. In addition, the method of evaluating transcription factors allowed us to obtain quantitative measurements.

Clinical outcome assessments, especially using left and right ventricular function up to 5 days after surgery, may be a good way to follow-up ventricular functions in post-ischaemia re-perfusion.

Conclusions

In children undergoing tetralogy of Fallot surgery, oral curcumin supplementation at a dose of 45 mg/day for 14 days pre-operatively greatly reduced active c-Jun N-terminal kinase protein from the pre-ischaemia to the re-perfusion phases, suppressed caspase-3 expression in the ischaemia phase, and improved myocardial performance, body temperature, and oxygen saturation at the 6-hour stage. Curcumin administration might be considered as a standard protocol before tetralogy of Fallot correction to suppress apoptosis responses due to ischaemia re-perfusion injury. Further studies are warranted to evaluate the potential of curcumin to improve antioxidant levels in children requiring this surgical procedure.

Acknowledgement

Financial Support

This research received no spesific grant from any funding agency, commercial, or not-for-profit sectors.

References

1. Perry LW, Neill CA, Ferencz C. EUROCAT Working Party on Congenital Heart Disease: Perspective in Pediatric Cardiology. Epidemiology of Congenital Heart Disease, the Baltimore-Washington Infant Study 1981–89. Futura, Armonk, New York, 1993: 33–62.
2. Goldhaber JI, Weiss JN. Oxygen free radicals and cardiac reperfusion abnormalities. Hypertension 1992; 20: 118–127.
3. Rubiana S, Ponisih. Procalcitonin and pathogens growth in febrile post pediatric cardiac surgery. APPCS 2014; 7 (Suppl 1).
4. Dhalla N, Elmoselhi A, Hatac T, Makino N. Status of myocardial antioxidants in ischemia–reperfusion injury. Cardiovasc Res 2000; 47: 446–456.
5. Armstrong SC. Protein kinase activation and myocardial ischemia/reperfusion injury. Cardiovasc Res 2004; 61: 427–436.
6. Rafiee P, Shi Y, Kong X, et al. Activation of protein kinases in chronically hypoxic infant human and rabbit hearts: role in cardioprotection. Circulation 2002; 106: 239–245.
7. Alwi I. Correlation of Metabolic Factors with Inflammatory Response in Acute Coronary Syndrome in Type 2 Diabetes Mellitus. Dissertation, University of Indonesia, Jakarta, 2006.
8. Wills ED. Evaluation of lipid peroxidation on lipids and biological membranes. In Snell K, Mullock B (eds). Biochemical Toxicology. A Practical Approach. IRI Press Limited, Oxford, 1987: 127–152.
9. Devasagayam TPA, Boloor KK, Ramasarma T. Methods for estimating lipid peroxidation: an analysis of merits and demerits. Indian J Biochem Bio 2003; 40: 300–308.
10. Ellman DL. Tissue sulfhydril groups. Arch Biochem Biophys 1959; 82: 70–79.
11. Peterson GL. A simplification of protein assay method of Lowry et al. Which is more generally applicable? Anal Biochem 1977; 83: 345–356.
12. Suskind DL, Wahbeh G, Burpee T, Cohen M, Christie D, Weber W. Tolerability of curcumin in pediatric inflammatory bowel disease: a forced-dose titration study. J Pediatr Gastroenterol Nutr 2013; 56: 277–279.
13. Alwi I, Santoso T, Suyono S, et al. The effect of curcumin on lipid level in patients with acute coronary syndrome. Acta Med Indones 2008; 40: 201–210.
14. Pirinccioglu AG, Alyan O, Kizil G, Kangin M, Beyazit N. Evaluation of oxidative stress in children with congenital heart defects. Pediatr Int 2012; 54: 94–98.
15. Ozer M, Parlakpinar H, Cigremis Y, Ucar M, Vardi N, Acet A. Ischemia-reperfusion leads to depletion of glutathione content and augmentation of malondialdehyde production in the rat heart from overproduction of oxidants: can caffeic acid phenethyl ester (CAPE) protect the heart? Mol Cell Biochem 2005; 273: 169–175.
16. Namratha K, Shenai P, Chatra L, Rao PK, Veena KM, Prabhu RV. Antioxidant and anticancer effects of curcumin – a review. J Contemp Med 2013; 3: 136–143.
17. Haramaki N, Steward DB, Aggrawal S, Ikeda H, Reznick AZ, Packer L. Networking antioxidants in the isolated rat heart are selectively depleted by ischemia-reperfusion. Free Radic Biol Med 1998; 25: 329–339.
18. Itokawa H, Shi Q, Akiyama T, Morris-Natschke SL, Lee KH. Recent advances in the investigation of curcuminoids. Chin Med 2008; 3: 1–13.
19. Reddy S, Osorio JC, Deque AM, et al. Failure of right ventricle adaptation in the children with tetralogy of Fallot. Circulation 2006; 114 (Suppl I): I37–I42.
20. Avci G, Kadioglu H, Sehirli AO, et al. Curcumins protects against ischemia/reperfusion injury in skeletal muscle. J Surg Res 2012; 172: e39–e46.
21. Cheng H, Liu W, Ai X. Protective effect of curcumin on myocardial ischemia reperfusion injury in rats. Zhong Yao Cai 2005; 28: 920–922; (Abstract in Chinese).
22. Ambrosio G, Flaherty JT, Duilio CD. Oxygen radicals generated at reflow induce peroxidation of membrane lipids in reperfusion hearts. J Clin Invest 1991; 87: 2056–2066.
23. Oeckinghaus A, Ghosh S. The NF-κB Family of transcription factors and its regulation. Cold Spring Harb Perspect Biol 2009; 1: 1–15.
24. Widmann C, Gibson S, Jarpe MB, Johnson GL. Mitogen-activated protein kinase: conservation of a three- kinase module from yeast to human. Physiol Rev 1999; 79: 143–180.
25. Gerits N, Kostenko S, Moens U. In vivo functions of mitogen-activated protein kinases: conclusions from knock-in and knock-out mice. Transgenic Res 2007; 16: 281–314.
26. Krijnen PAJ, Nijmeijer R, CJLM Meijer, Visser CA, Hack CE, Niessen HWM. Apoptosis in myocardial ischemia and infarction. J Clin Pathol 2002; 55: 801–811.
27. Haunstetter A, Izumo S. Apoptosis: basic mechanisms and implications for cardiovascular disease. Circ Res 1998; 82: 1111–1129.
28. Milano G, Morel S, Bonny C, et al. A peptide inhibitor of c-Jun NH2-terminal kinase reduces myocardial ischemia- reperfusion injury and infarct size in vivo. Am J Physiol Heart Circ Physiol 2007; 292: 1828–1835.
29. Yue TL, Wang C, Gu JL, et al. Inhibition of extracellular signal-regulated kinase enhances ischemia/reoxygenation-induced apoptosis in cultured cardiomyocyte and exaggerates reperfusion injury in isolated perfused heart. Circ Res 2000; 86: 692–699.
30. Shao Z, Bhattacharya K, Hsich E, et al. c-Jun N-terminal kinases mediate reactivation of Akt and cardiomyocyte survival after hypoxic injury in vitro and in vivo. Circ Res 2006; 98: 111–118.
31. Fiorillo C, Becatti M, Pensalini A, et al. Curcumin protects cardiac cells against ischemia-reperfusion injury: effects on oxidative stress, NFκB, and JNK pathways. Free Radic Biol Med 2008; 45: 839–846.
32. Literat A, Su F, Norwicki M, et al. Regulation of pro-inflammatory cytokine expression by curcumin in hyaline membrane disease (HMD). Life Sci 2001; 70: 253–267.
33. Mukhopadhyay A, Basu N, Ghatak N. Anti-inflammatory and irritant activities of curcumin analogues in rats. Agents Actions 1982; 12: 508–515.
34. Hindman BJ. Emboli, inflammation, and CNS impairement: an overview. Heart Surg Forum 2002; 5: 249–253.
35. Mitchell JD, Grocott HP, Phillips-Bute B, Mathew JP, Newman MF, Bar-Yosef S. Cytokine secretion after cardiac surgery and its relationship to postoperative fever. Cytokine 2007; 38: 37–42.
36. Butts RJ, Scheurer MA, Atz AM, et al. Comparison of maximum vasoactive inotropic score and low cardiac output syndrome as markers of early postoperative outcomes after neonatal cardiac surgery. Pediatr Cardiol 2012; 33: 633–638.
37. Top APC, Tasker RC, Ince C. The micro circulation of clinically ill pediatric patients. Circ med 2011; 15: 213–219.
38. Temsah RM, Netticadan T, Chapman D, Takeda S, Mochizuki S, Dhalla NS. Alterations in sarcoplasmic reticulum function and gene expression in ischemia-reperfused rat heart. Am J Physiol 1999; 277: 584–594.
39. Kim YS, Park HJ, Joo SY, et al. The protective effect of curcumin on myocardial ischemia-reperfusion injury. Korean Circ J 2008; 38: 353–369.
40. Wang NP, Wang ZF, Tootle S, Philip T, Zhao ZQ. Curcumin promotes cardiac repair and ameliorates cardiac dysfunction following myocardial infarction. Br J Pharmacol 2012; 167: 1550–1562.