BJR

The efficacy of statins in preventing glucocorticoid-related osteonecrosis in animal models

A META-ANALYSIS

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Objectives
The primary purpose of this meta-analysis was to determine whether statin usage could reduce the risk of glucocorticoid-related osteonecrosis in animal models.

Methods
A systematic literature search up to May 2015 was carried out using the PubMed, Ovid, EBM reviews, ISI Web of Science, EBSCO, CBM, CNKI databases with the term and boolean operators: statins and osteonecrosis in all fields. Risk ratio (RR), as the risk estimate of specific outcome, was calculated along with 95% confidence intervals (CI). The methodological quality of individual studies was assessed using a quantitative tool based on the updated Stroke Therapy Academic Industry Roundtable (STAIR) recommendations.

Results
A total of 11 eligible studies were included according to predetermined criteria. The pooled data demonstrated that animals with statin usage, either alone or combined with other treatments, were at a decreased risk of developing glucocorticoid-related osteonecrosis (RR = 2.06, 95% confidence interval (CI) 1.71 to 2.50). Moreover, subgroup analysis revealed that compared with statins alone, statins combined with other treatments significantly decreased the risk of osteonecrosis (RR = 1.23, 95% CI 1.02 to 1.47). However, we could find no significant risk difference for different gender, or for different time points.

Conclusions
The present study suggests that statins combined with other treatments are efficient in preventing the development of glucocorticoid-related osteonecrosis in animals. These results might shed light on clinical practice when glucocorticoids are prescribed, and could be further investigated in high-quality clinical trials.

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Article focus
■ Our study aimed to determine whether statin usage reduces the risk of glucocorticoid (GC)-related osteonecrosis (ON) in animal models.

Key messages
■ Our results suggest that statin usage combined with other treatments could reduce the risk of developing GC-related ON in animal models.

Strengths and limitations
■ This is the first meta-analysis to review the efficacy of statins in preventing GC-induced ON in animal models.

■ No distinction was made for animal models and the type or dose of statins and GCs.

Introduction
Osteonecrosis (ON) is a common, progressive and devastating disease with an insidious onset. ON presents without specific clinical symptoms and signs and most commonly affects young adults in the third and fourth decade of their life.[1-3] Management options[1,2] for ON vary from joint salvaging procedures to joint replacement, and are based on the stages described by the Association of Research Circulation Osseous (ARCO) classification. Conservative treatment,

Table I. Criteria for selection of studies in meta-analysis

Criteria for inclusion	Criteria for exclusion
Animal models induced by GCs	Animal models induced by other factors
The exposure of interest was the usage of statin	Studies without a control group
The outcome was incidence of ON	Data unable to be extracted
Studies providing sufficient information to calculate risk estimate and corresponding 95% confidence interval	

Table II. Basic characteristics of included studies

Study ID	Study location	Species	n (C)	n (E)	Gender	Age (wks)	Weight
Li et al 2014[23]	China	Rabbit	15	15	Female	24 to 28	2.5 kg to 3.1 kg
Xie et al 2013[28]	China	Rat	30	60	Mixed	8	250 g to 280 g
Nozaki et al 2012[13]	Japan	Rat	23	15	Male	13	NR
Li et al 2011[27]	China	Rabbit	16	16	Female	28 to 32	2.8 kg to 3.4 kg
Kang et al 2010[14]	China	Rabbit	24	52	Male	28 to 32	2.8 kg to 3.4 kg
Zeng et al 2009[26]	China	Rabbit	9	20	Mixed	Adult	2.5 kg to 3.0 kg
Iwakiri et al 2008[15]	Japan	Rabbit	30	30	Female	28 to 32	3.1 kg to 4.2 kg
Pengde et al 2008[16]	China	Rabbit	26	25	Male	28 to32	2.8 kg to 3.4 kg
Nishida et al 2008[17]	Japan	Rabbit	30	35	Male	28 to 32	NR
Kang et al 2007[29]	China	Rabbit	16	32	Mixed	28 to 32	2.7 kg to 3.3 kg
Cui et al 1997[18]	United States	Chicken	25	10	Female	Adult	NR

n (C), number of animals in control group; n (E), number of animals in experimental group; NR, not reported

Table III. Characteristics of animal models of steroid-induced osteonecrosis

Study ID	Animal models		
	Steroids exposure	Dose (mg/kg)	Route of delivery
Li et al 2014[23]	MPSL (+ET)	20 (10 µg), once	i.m. (left gluteus)
Xie et al 2013[28]	MPSL	20, daily, 4 wks	i.p.
Nozaki et al 2012[13]	MPSL	4 mg in total	s.c. (back)
Li et al 2011[27]	MPSL	20, once	i.m. (right gluteus)
Kang et al 2010[14]	MPSL	20, once	i.m. (right gluteus)
Zeng et al 2009[26]	DSP	2.5, NR	i.m.
Iwakiri et al 2008[15]	MPSL	20, once	i.m. (right gluteus)
Pengde et al 2008[16]	MPSL	20, once	i.m. (right gluteus)
Nishida et al 2008[17]	MPSL	20, once	i.m. (right gluteus)
Kang et al 2007[29]	MPSL	20, once	i.m. (right gluteus)
Cui et al 1997[18]	MPSL	3, weekly, 12 wks	i.m.

MPSL, methylprednisolone; DSP, dexamethasone sodium phosphate; ET, endotoxin; i.m., intramuscular; i.v., intravenous; i.p., intraperitoneal; s.c., subcutaneous injection; NR, not reported

represented by core decompression, may be efficient in the early stages and in small lesions of osteonecrosis (stages I, II). However, concerns have been raised due to the potential for core decompression to weaken the cancellous bone within and adjacent to the necrotic zones.[4] With respect to joint replacement surgeries, approximately 10 000 to 20 000 new cases of ON are diagnosed in the United States every year, and it is estimated that 5% to 12% of total hip arthroplasties are performed every year in order to treat this disease.[1,2]

Glucocorticoid (GC) usage is the leading cause of non-traumatic ON of the femoral head.[5] ON develops in 9% to 40% of patients who receive high-dose or long-term steroid therapy.[6] Despite the strong association of GC with ON, the underlying mechanisms of ON have been unclear. Several hypotheses have been introduced that explain the mechanism of GC-induced ON. The proposed pathogenesis includes lipid metabolism disturbance,

apoptosis, increased oxidative stress and disturbances of the coagulation-fibrinolysis system due to steroid hormones.[1-3,7,8] In particular, intraosseous hypertension, intravenous fat embolisms, and compression of vessels by progressive accumulation of marrow fat store, are commonly accepted theories.[9] Based on these findings, an increasing number of studies have been initiated to explore the effects of lipid-lowering agents on preventing ON.[9-20]

Statins (3-hydroxymethyl-3-glutaryl-CoA (HMG-CoA) reductase inhibitors), widely used for the treatment of hyperlipidemia as well as for preventing coronary artery diseases,[21,22] have been likewise an attractive candidate for prevention of GC-induced ON. Beneficial effects do not only result from lowering cholesterol level, but also from pleiotropic effects including improvement of endothelial dysfunction, antioxidant effects, reduction of platelet activity and decreased bone cell apoptosis.[21-23]
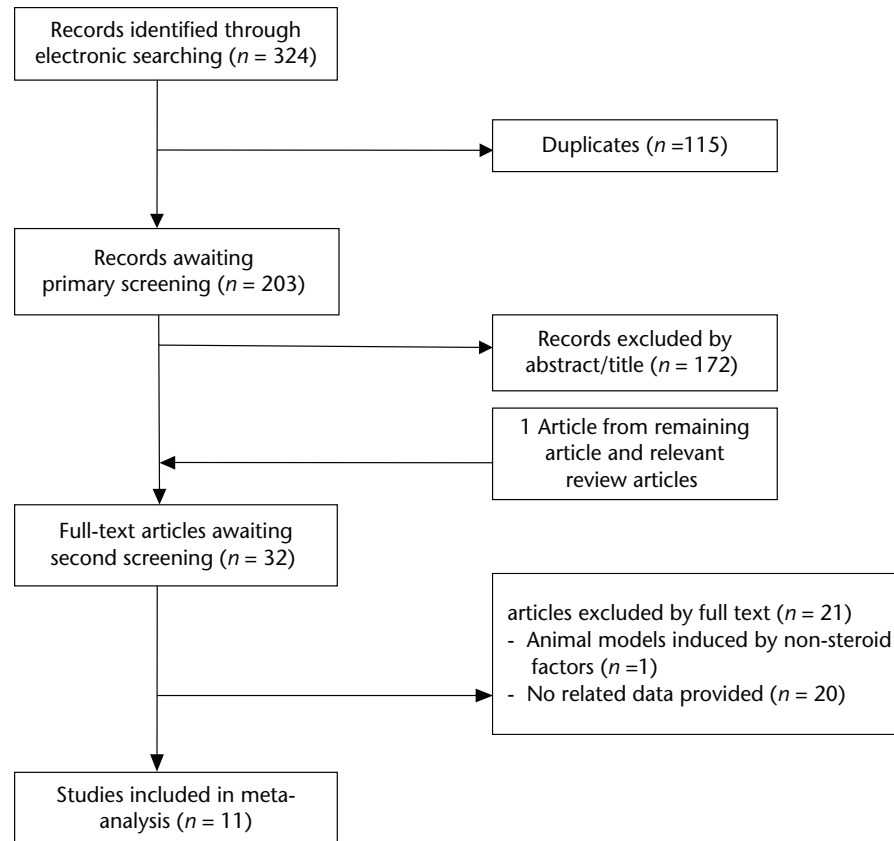
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│ Records awaiting            │
│ primary screening (n = 203) │─────────────┐
└─────────────────────────────┘             ▼
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              │                     │ abstract/title       │
              │                     │ (n = 172)            │
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              │          ◄──────────│ 1 Article from       │
              │                     │ remaining article    │
              │                     │ and relevant review  │
              │                     │ articles             │
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│ Full-text articles awaiting │
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              │                     │ articles excluded by full     │
              │                     │ text (n = 21)                 │
              │                     │ - Animal models induced by    │
              │                     │   non-steroid factors (n =1)  │
              │─────────────────────▶ - No related data provided    │
              │                     │   (n = 20)                    │
              ▼                     └───────────────────────────────┘
┌─────────────────────────────┐
│ Studies included in meta-   │
│ analysis (n = 11)           │
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Fig. 1

Flow diagram showing the process of article selection for meta-analysis.

Table IV. Characteristics of statin intervention of included studies

Study ID	Types	Statins administration			
		Dose(mg/kg.d,)	Duration (wks)	Route of delivery	Treatment point before steroid
Li et al 2014[23]	atorvastatin	2.5	8	Food admixture	0 wk
Xie et al 2013[28]	lovastatin	25	14	Gavage	2 wks
Nozaki et al 2012[13]	pravastatin	15	4	Drinking water	2 wks
Li et al 2011[27]	pravastatin	2.5	12	Food admixture	0 wk
Kang et al 2010[14]	lovastatin	5	14	Food admixture	2 wks
Zeng et al 2009[26]	simvastatin	20mg, daily	NR	PO	0 wk
Iwakiri et al 2008[15]	pravastatin/simvastatin	2/5	6/6	IV/IV	3 wks/3 wks
Pengde et al 2008[16]	lovastatin	5	14	Food admixture	2 wks
Nishida et al 2008[17]	pravastatin	0.7	4	IV	2 wks
Kang et al 2007[29]	lovastatin	300	14	Food admixture	2 wks
Cui et al 1997[18]	lovastatin	20 mg, daily	NR	PO	0 wk

PO, peros; IV, intravenous; NR, not reported

These various effects of statins may play an important role in the prevention of GC-induced ON. Previous research studies[14-16] have shown serum lipid levels were significantly lower in the statin group than in the control group, which received steroids only.

Interestingly, Iwakirietal et al[15] have shown that increased CYP3A activity owing to statins is a possible mechanism for the protective effect, given the fact that extrinsic glucocorticoids are made inactivate predominantly by hepatic CYP3A. Histological examination[14-16] revealed that rabbits treated with steroids and statins maintained more physiological bone marrow fat cell size and fraction of marrow filled by fat. *In vitro* and *in vivo* studies[9,18] demonstrated that statin acts on bone marrow mesenchymal stem cells and modulate their differentiation by enhancing osteogenesis through increasing expression of *Cbfa1/Runx2* improving activity of the osteocalcin promoter, and inhibiting the adipogenesis through decreasing expression of the adipocyte-specific genes *PPARγ2* (fat cell transcription factor) and *422aP* (fat-specific). Statins

Table V. The methodological quality of individual study

Study ID	(1)	(2)	(3)	(4)	(5)	(6)	(7)	Score
Li et al 2014[23]		*	*		*			3
Xie et al 2013[28]		*			*	*		3
Nozaki et al 2012[13]	*	*			*	*	*	5
Li et al 2011[27]		*			*			2
Kang et al 2010[14]		*			*	*		3
Zeng et al 2009[26]		*			*			2
Iwakiri et al 2008[15]		*			*	*	*	4
Pengde et al 2008[16]		*			*	*		3
Nishida et al 2008[17]	*	*			*			3
Kang et al 2007[29]		*			*			2
Cui et al 1997[18]		*			*			2

*One score
Studies fulfilling the criteria of (1) sample size calculation; (2) inclusion and exclusion criteria; (3) randomisation; (4) allocation concealment; (5) reporting of animals excluded from analysis; (6) blinded assessment of ON; (7) reporting potential conflicts of interest and study funding

Table VI. Evaluation of osteonecrosis of included studies

Study ID	Diagnosis of osteonecrosis		
	Unilateral or bilateral	Sample from	Methods
Li et al 2014[23]	Bilateral	Femoral head	Radiological examination
Xie et al 2013[28]	NR	Femoral head	Histological examination
Nozaki et al 2012[13]	Bilateral	Proximal femur	Histological examination
Li et al 2011[27]	Unilateral	Femoral head	Histological examination
Kang et al 2010[14]	Bilateral	Femur and humerus*	Histological examination
Zeng et al 2009[26]	Bilateral	Femoral heads	Radiological examination
Iwakiri et al 2008[15]	Bilateral	Proximal femur	Histological examination
Pengde et al 2008[16]	Bilateral	Femur and humerus*	Histological examination
Nishida et al 2008[17]	Bilateral	Femur and humerus*	Histological examination
Kang et al 2007[29]	Bilateral	Proximal femur	Histological examination
Cui et al 1997[18]	Bilateral	Femoral head	Histological examination

*The whole area of the proximal one third and distal condyles of both the femur and the humerus
NR, not reported

Table VII. Outcomes of each included study

Study ID	Animals treated with statin and steroid		Animals treated with steroid alone	
	Osteonecrosis	Total	Osteonecrosis	Total
Li et al 2014[23]	4	15	7	15
Xie et al 2013[28]	19	60	17	30
Nozaki et al 2012[13]	11	15	23	23
Li et al 2011[27]	5	16	10	16
Kang et al 2010[14]	13	52	16	24
Zeng et al 2009[26]	1	20	3	9
Iwakiri et al 2008[15]	9	30	25	30
Pengde et al 2008[16]	9	25	18	26
Nishida et al 2008[17]	13	35	21	30
Kang et al 2007[29]	8	32	11	16
Cui et al 1997[18]	0	10	14	25

are also associated with an elevated bone morphogenetic protein-2 gene expression, alkaline phosphatase activity, matrix mineralisation, and enhanced osteogenesis by the bone cells *in vitro*.[9] Although several animal and limited clinical studies indicated that statins may have a protective role against ON a recent large-scale cohort study[10] revealed that ON-free survival was similar in renal transplantation patients with and without statin exposure.

It is therefore not clear if statins could effectively prevent GC-related ON. Therefore, the primary purpose of this meta-analysis is to determine whether statin usage reduces the risk of GC-related ON in animal models.

Materials and Methods

Search strategy. An electronic search was conducted online to identify relevant studies up to May 2015, using the PubMed, Ovid MEDLINE(R) (1946 to present with daily update), all EBM reviews, ISI Web of Science, Academic Search Premier and MEDLINE in EBSCO, China Biological Medicine Database, and China National Knowledge Infrastructure databases with the following terms and boolean operators: statins and osteonecrosis in all fields. In addition, bibliographies of retrieved articles were searched by hand for further pertinent studies. Furthermore, we contacted
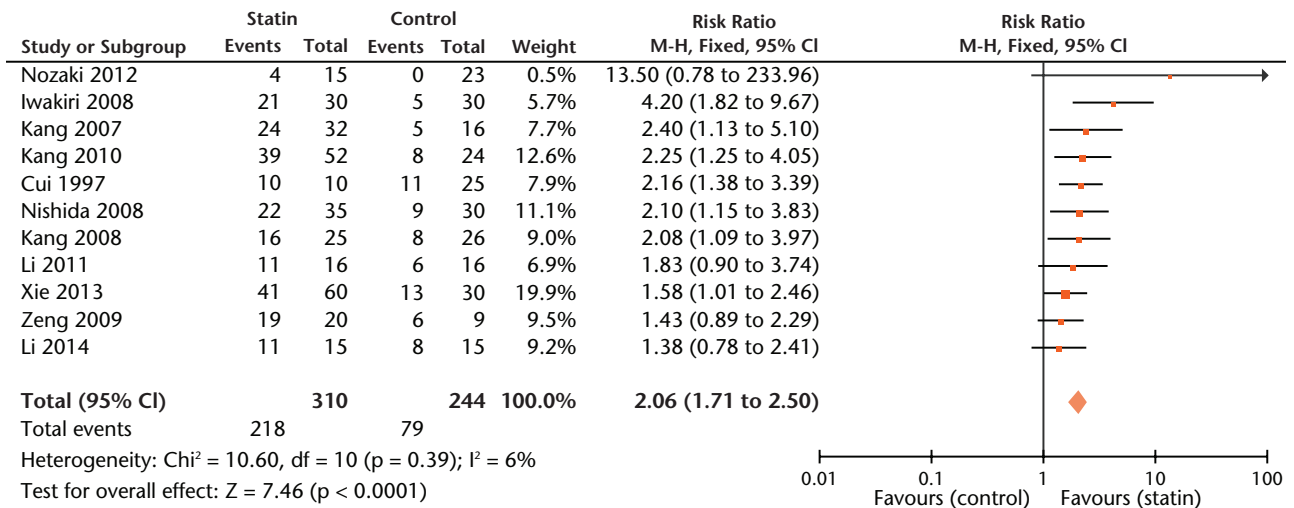
Study or Subgroup	Statin Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Nozaki 2012	4	15	0	23	0.5%	13.50 (0.78 to 233.96)
Iwakiri 2008	21	30	5	30	5.7%	4.20 (1.82 to 9.67)
Kang 2007	24	32	5	16	7.7%	2.40 (1.13 to 5.10)
Kang 2010	39	52	8	24	12.6%	2.25 (1.25 to 4.05)
Cui 1997	10	10	11	25	7.9%	2.16 (1.38 to 3.39)
Nishida 2008	22	35	9	30	11.1%	2.10 (1.15 to 3.83)
Kang 2008	16	25	8	26	9.0%	2.08 (1.09 to 3.97)
Li 2011	11	16	6	16	6.9%	1.83 (0.90 to 3.74)
Xie 2013	41	60	13	30	19.9%	1.58 (1.01 to 2.46)
Zeng 2009	19	20	6	9	9.5%	1.43 (0.89 to 2.29)
Li 2014	11	15	8	15	9.2%	1.38 (0.78 to 2.41)
Total (95% CI)		**310**		**244**	**100.0%**	**2.06 (1.71 to 2.50)**
Total events	218		79			

Heterogeneity: Chi² = 10.60, df = 10 (p = 0.39); I² = 6%
Test for overall effect: Z = 7.46 (p < 0.0001)

Fig. 2

Forest plot showing the overall risk estimate of osteonecrosis between groups with or without statins (M-H, Mantel–Haenszel; CI, confidence interval; df, degrees of freedom). Risk ratio (RR) on the left axis indicates statin usage decreases the risk of osteonecrosis compared with the control group, whereas RR greater than 1 indicates animals with statin usage are at increased risk of osteonecrosis. The 95% CI reveals that the result is statistically significant when "1" is not included in the interval.

Study or Subgroup	Statin Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.5.1 3 points or more						
Nozaki 2012	4	15	0	23	0.5%	13.50 (0.78 to 233.96)
Iwakiri 2008	21	30	5	30	5.7%	4.20 (1.82 to 9.67)
Kang 2010	39	52	8	24	12.6%	2.25 (1.25 to 4.05)
Nishida 2008	22	35	9	30	11.1%	2.10 (1.15 to 3.83)
Kang 2008	16	25	8	26	9.0%	2.08 (1.09 to 3.97)
Xie 2013	41	60	13	30	19.9%	1.58 (1.01 to 2.46)
Li 2014	11	15	8	15	9.2%	1.38 (0.78 to 2.41)
Subtotal (95% CI)		**232**		**178**	**68.1%**	**2.13 (1.67 to 2.70)**
Total events	154		51			

Heterogeneity: Chi² = 8.27, df = 6 (p = 0.22); I² = 27%
Test for overall effect: Z = 6.16 (p < 0.00001)

1.5.2 less than 3 points						
Kang 2008	24	32	5	16	7.7%	2.40 (1.13 to 5.10)
Cui 1997	10	10	11	25	7.9%	2.16 (1.38 to 3.39)
Li 2011	11	16	6	16	6.9%	1.83 (0.90 to 3.74)
Zeng 2009	19	20	6	9	9.5%	1.43 (0.89 to 2.29)
Subtotal (95% CI)		**78**		**66**	**31.9%**	**1.93 (1.42 to 2.61)**
Total events	64		28			

Heterogeneity: Chi² = 2.15, df = 3 (p = 0.54); I² = 0%
Test for overall effect: Z = 4.25 (p < 0.0001)

Total (95% CI)		**310**		**244**	**100.0%**	**2.06 (1.71 to 2.50)**
Total events	218		79			

Heterogeneity: Chi² = 10.60, df = 10 (p = 0.39); I² = 6%
Test for overall effect: Z = 7.46 (p < 0.00001)
Test for subgroup differences: Chi² = 0.25, df = 1 (p = 0.62); I² = 0%
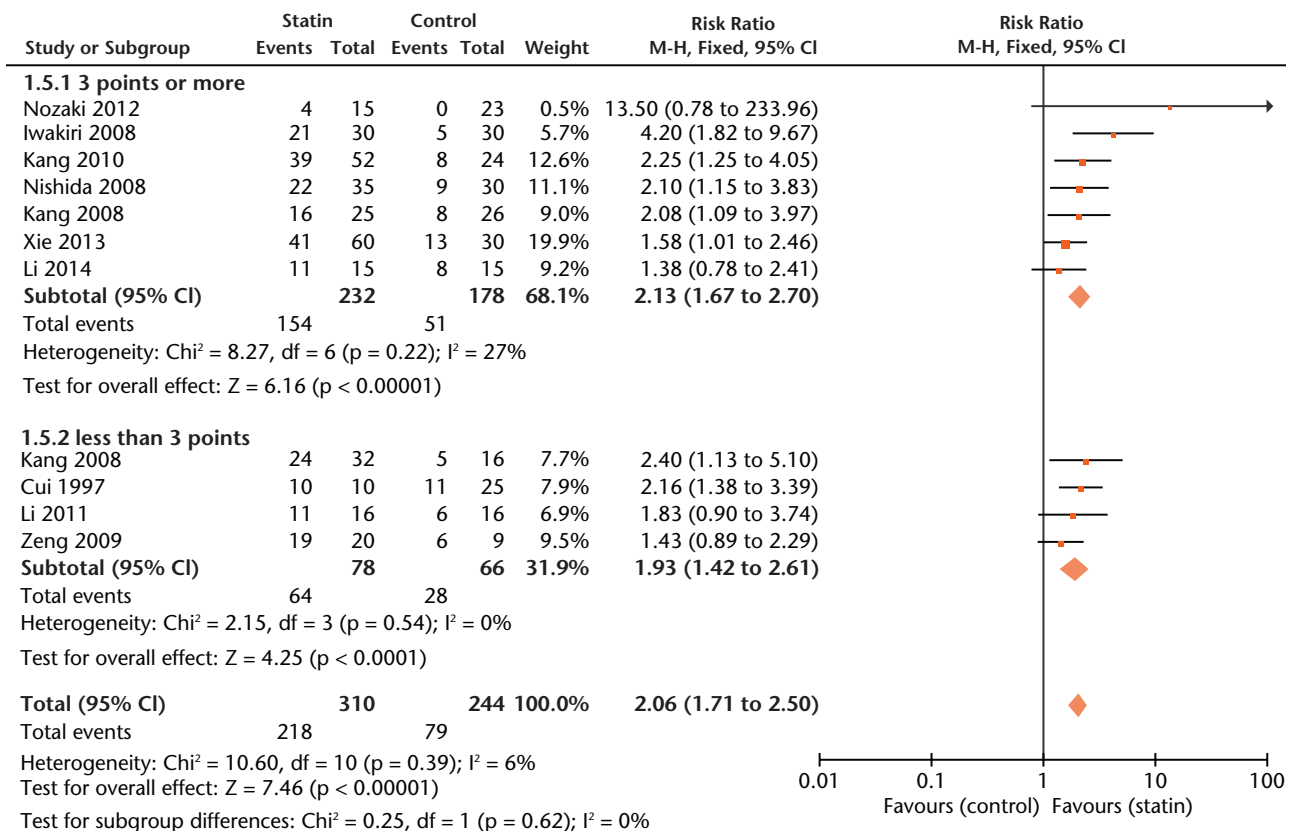

Fig. 3

Forest plot showing subgroup analysis based on study quality score (M-H, Mantel–Haenszel; CI, confidence interval; df, degrees of freedom). Risk ratio (RR) on the left axis indicates statin usage decreases the risk of osteonecrosis compared with the control group, whereas RR greater than 1 indicates animals with statin usage are at increased risk of osteonecrosis. The 95% CI reveals that the result is statistically significant when "1" is not included in the interval, and *vice versa*.

the authors of the studies to collect raw data and complete the search strategy when possible. The two investigators independently selected potential eligible studies according to predetermined criteria (Table I). No language restrictions were imposed. Any discrepancy between them was resolved by consensus. No
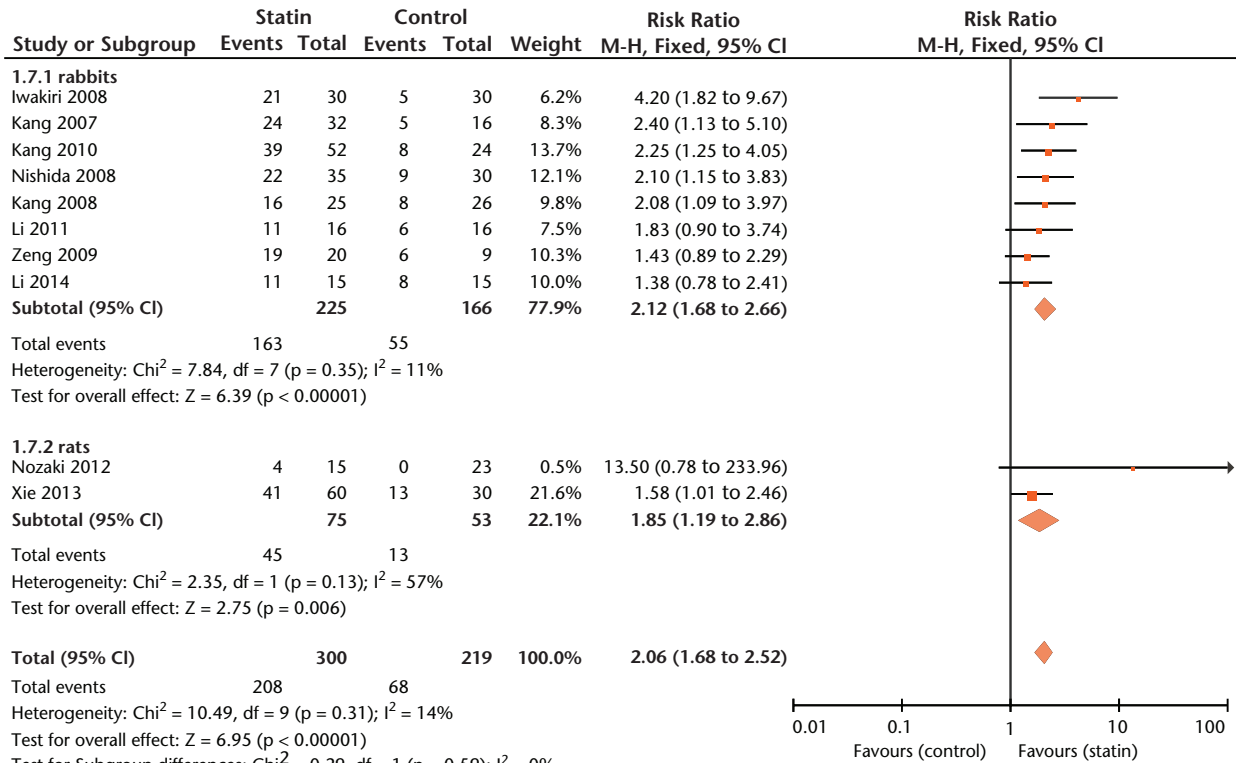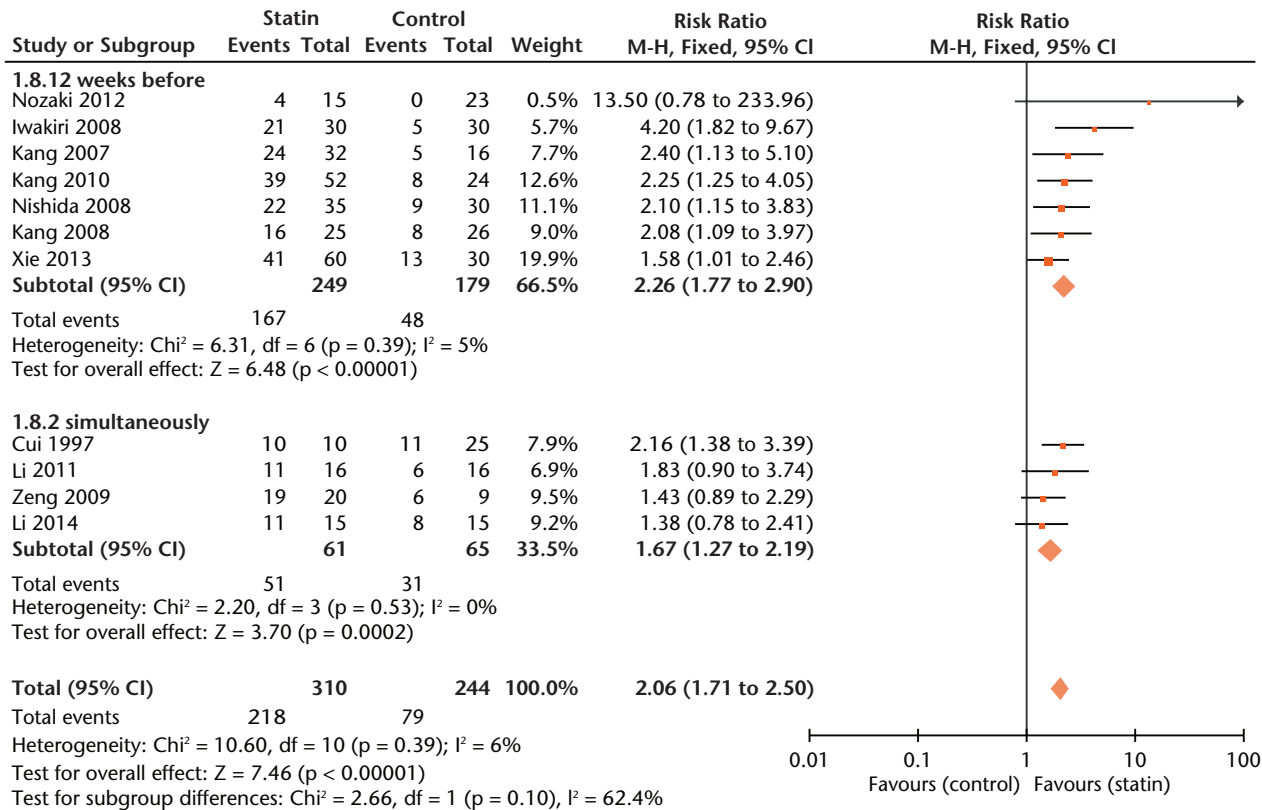
Study or Subgroup	Statin Events	Statin Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.7.1 rabbits						
Iwakiri 2008	21	30	5	30	6.2%	4.20 (1.82 to 9.67)
Kang 2007	24	32	5	16	8.3%	2.40 (1.13 to 5.10)
Kang 2010	39	52	8	24	13.7%	2.25 (1.25 to 4.05)
Nishida 2008	22	35	9	30	12.1%	2.10 (1.15 to 3.83)
Kang 2008	16	25	8	26	9.8%	2.08 (1.09 to 3.97)
Li 2011	11	16	6	16	7.5%	1.83 (0.90 to 3.74)
Zeng 2009	19	20	6	9	10.3%	1.43 (0.89 to 2.29)
Li 2014	11	15	8	15	10.0%	1.38 (0.78 to 2.41)
Subtotal (95% CI)		225		166	77.9%	2.12 (1.68 to 2.66)
Total events	163		55			

Heterogeneity: Chi^2 = 7.84, df = 7 (p = 0.35); I^2 = 11%
Test for overall effect: Z = 6.39 (p < 0.00001)

Study or Subgroup	Statin Events	Statin Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.7.2 rats						
Nozaki 2012	4	15	0	23	0.5%	13.50 (0.78 to 233.96)
Xie 2013	41	60	13	30	21.6%	1.58 (1.01 to 2.46)
Subtotal (95% CI)		75		53	22.1%	1.85 (1.19 to 2.86)
Total events	45		13			

Heterogeneity: Chi^2 = 2.35, df = 1 (p = 0.13); I^2 = 57%
Test for overall effect: Z = 2.75 (p = 0.006)

	Statin Total	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Total (95% CI)	300	219	100.0%	2.06 (1.68 to 2.52)
Total events	208	68		

Heterogeneity: Chi^2 = 10.49, df = 9 (p = 0.31); I^2 = 14%
Test for overall effect: Z = 6.95 (p < 0.00001)
Test for Subgroup differences: Chi^2 = 0.29, df = 1 (p = 0.59); I^2 = 0%

Fig. 4

Forest plot showing subgroup analysis based on species (M-H, Mantel–Haenszel; CI, confidence interval; df, degrees of freedom). Risk ratio (RR) on the left axis indicates statin usage decreases the risk of osteonecrosis compared with the control group, whereas RR greater than 1 indicates animals with statin usage are at increased risk of osteonecrosis. The 95% CI reveals that the result is statistically significant when "1" is not included in the interval, and *vice versa*.
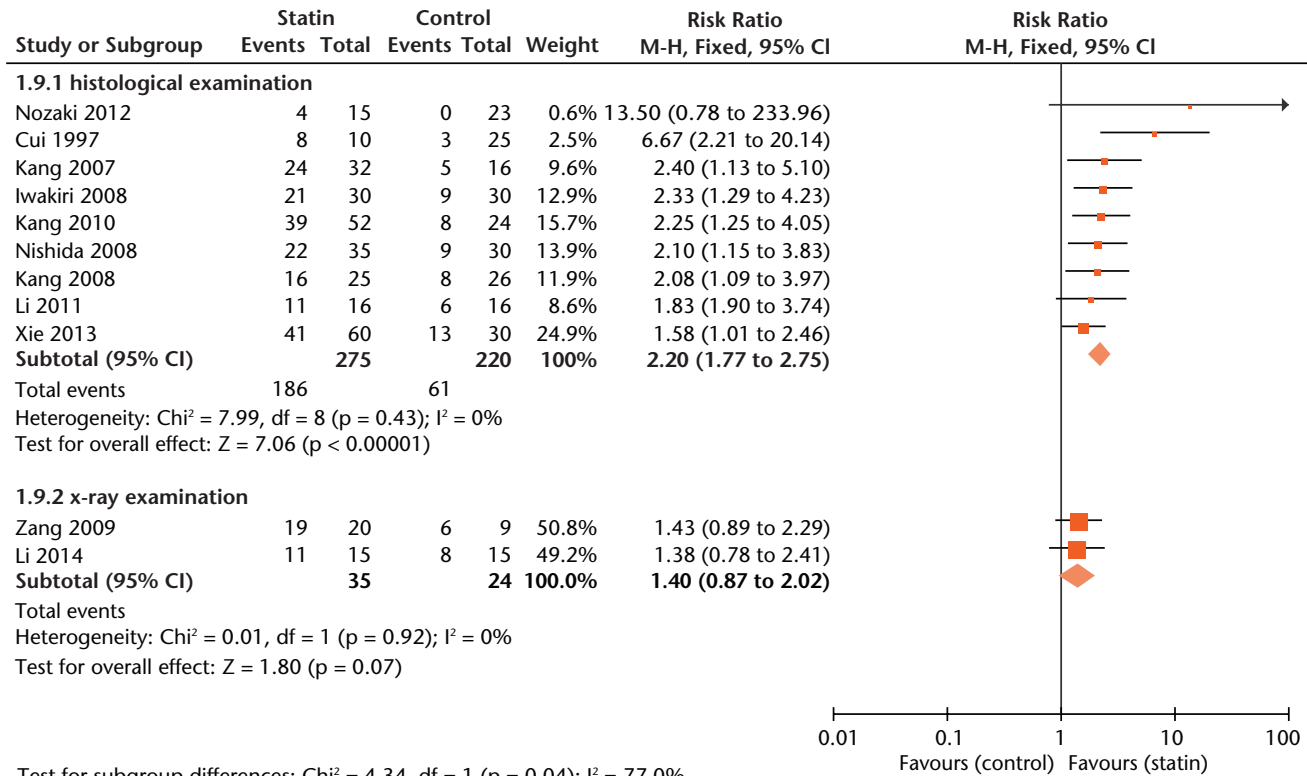
Study or Subgroup	Statin Events	Statin Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.8.12 weeks before						
Nozaki 2012	4	15	0	23	0.5%	13.50 (0.78 to 233.96)
Iwakiri 2008	21	30	5	30	5.7%	4.20 (1.82 to 9.67)
Kang 2007	24	32	5	16	7.7%	2.40 (1.13 to 5.10)
Kang 2010	39	52	8	24	12.6%	2.25 (1.25 to 4.05)
Nishida 2008	22	35	9	30	11.1%	2.10 (1.15 to 3.83)
Kang 2008	16	25	8	26	9.0%	2.08 (1.09 to 3.97)
Xie 2013	41	60	13	30	19.9%	1.58 (1.01 to 2.46)
Subtotal (95% CI)		249		179	66.5%	2.26 (1.77 to 2.90)
Total events	167		48			

Heterogeneity: Chi^2 = 6.31, df = 6 (p = 0.39); I^2 = 5%
Test for overall effect: Z = 6.48 (p < 0.00001)

Study or Subgroup	Statin Events	Statin Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.8.2 simultaneously						
Cui 1997	10	10	11	25	7.9%	2.16 (1.38 to 3.39)
Li 2011	11	16	6	16	6.9%	1.83 (0.90 to 3.74)
Zeng 2009	19	20	6	9	9.5%	1.43 (0.89 to 2.29)
Li 2014	11	15	8	15	9.2%	1.38 (0.78 to 2.41)
Subtotal (95% CI)		61		65	33.5%	1.67 (1.27 to 2.19)
Total events	51		31			

Heterogeneity: Chi^2 = 2.20, df = 3 (p = 0.53); I^2 = 0%
Test for overall effect: Z = 3.70 (p = 0.0002)

	Statin Total	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Total (95% CI)	310	244	100.0%	2.06 (1.71 to 2.50)
Total events	218	79		

Heterogeneity: Chi^2 = 10.60, df = 10 (p = 0.39); I^2 = 6%
Test for overall effect: Z = 7.46 (p < 0.00001)
Test for subgroup differences: Chi^2 = 2.66, df = 1 (p = 0.10), I^2 = 62.4%

Fig. 5

Forest plot showing subgroup analysis based on treatment time point (M-H, Mantel–Haenszel; CI, confidence interval; df, degrees of freedom). Risk ratio (RR) on the right axis indicates statin usage decreases the risk of osteonecrosis compared with the control group, whereas RR greater than 1 indicates animals with statin usage are at increased risk of osteonecrosis. The 95% CI reveals that the result is statistically significant when "1" is not included in the interval, and *vice versa*.

Study or Subgroup	Statin Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI	Risk Ratio M-H, Fixed, 95% CI
1.9.1 histological examination							
Nozaki 2012	4	15	0	23	0.6%	13.50 (0.78 to 233.96)	
Cui 1997	8	10	3	25	2.5%	6.67 (2.21 to 20.14)	
Kang 2007	24	32	5	16	9.6%	2.40 (1.13 to 5.10)	
Iwakiri 2008	21	30	9	30	12.9%	2.33 (1.29 to 4.23)	
Kang 2010	39	52	8	24	15.7%	2.25 (1.25 to 4.05)	
Nishida 2008	22	35	9	30	13.9%	2.10 (1.15 to 3.83)	
Kang 2008	16	25	8	26	11.9%	2.08 (1.09 to 3.97)	
Li 2011	11	16	6	16	8.6%	1.83 (1.90 to 3.74)	
Xie 2013	41	60	13	30	24.9%	1.58 (1.01 to 2.46)	
Subtotal (95% CI)		275		220	100%	2.20 (1.77 to 2.75)	
Total events	186		61				

Heterogeneity: Chi^2 = 7.99, df = 8 (p = 0.43); I^2 = 0%
Test for overall effect: Z = 7.06 (p < 0.00001)

1.9.2 x-ray examination							
Zang 2009	19	20	6	9	50.8%	1.43 (0.89 to 2.29)	
Li 2014	11	15	8	15	49.2%	1.38 (0.78 to 2.41)	
Subtotal (95% CI)		35		24	100.0%	1.40 (0.87 to 2.02)	
Total events							

Heterogeneity: Chi^2 = 0.01, df = 1 (p = 0.92); I^2 = 0%

Test for overall effect: Z = 1.80 (p = 0.07)

Test for subgroup differences: Chi^2 = 4.34, df = 1 (p = 0.04); I^2 = 77.0%

Fig. 6

Forest plot showing subgroup analysis based on measurement (M-H, Mantel–Haenszel; CI, confidence interval; df, degrees of freedom). Risk ratio (RR) on the left axis indicates statin usage decreases the risk of osteonecrosis compared with the control group, whereas RR greater than 1 indicates animals with statin usage are at increased risk of osteonecrosis. The 95% CI reveals that the result is statistically significant when "1" is not included in the interval, and *vice versa*.
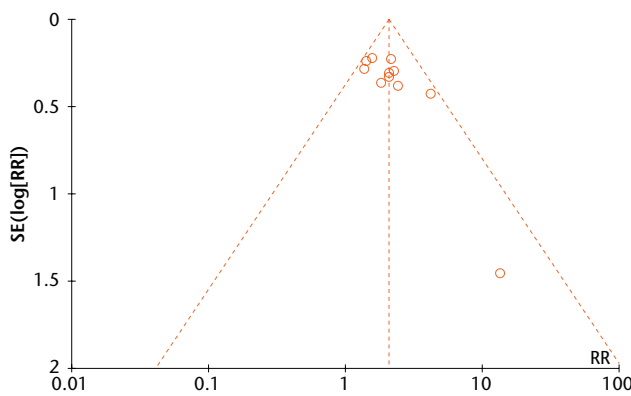
Fig. 7

Funnel plots evaluating publication bias among included studies.

distinction was made for animal models and the type or dose range of statins and GCs.

Data extraction. Data collection was conducted by two investigators independently and the result was checked by a third investigator. Discrepancies were settled by group discussion. Collected data included the first author's surname; publication year; study location; study design; sample size; type or dose range of statins and GC; duration of GC and statin usage; characteristics of animal models including species, animal age, weight, gender

and route of delivery in which GC and statins were administrated; the absolute number of ON cases in groups with or without statin exposure and the total number of animals with or without statin usage. When various statins or dosages were present in one study, data were analysed as a single group of those exposed to statins.

Assessment of methodological quality. Two reviewers independently assessed the methodology of the included articles with use of the updated Stroke Therapy Academic Industry Roundtable (STAIR) recommendations.[24] The methodological quality of individual study was scored against the following criteria: sample size calculation; inclusion and exclusion criteria; randomisation; allocation concealment; reporting of animals excluded from analysis; blinded assessment of osteonecrosis; reporting potential conflicts of interest and study funding. Each item was allocated one point for a quantitative appraisal of overall quality of the individual studies. Each study was given a quality score out of a possible total of seven points, and the group median was calculated.

Statistical analysis. The risk ratio (RR) was calculated by two investigators using the Cochrane review manager software (Version 5.3, Cochrane Collaboration, Oxford, United Kingdom). RR was calculated along with 95% confidence intervals (CI). Assessment of heterogeneity of included studies was conducted using Q and I^2 statistics.

Heterogeneity was not considered present with a p-value ≥ 0.1. Subgroup analyses were performed where applicable. Publication bias was accessed by funnel plots, using Egger's tests.[25] Significance was considered when a p-value was < 0.05.

Results

Search results and study characteristics. According to the aforementioned specific criteria, 11 eligible studies,[13-18,23,26-29] published between 1997 and 2014 were included in our meta-analysis. Figure 1 shows the detailed process of study selection. In total, we included 554 animals consisting of 391 rabbits, 128 rats and 35 chickens. Animal model ON was induced using steroid alone in all studies except for one,[23] which combined steroid with endotoxin. The study conducted by Nozaki et al[13] consisted of rats with a 50% incidence of spontaneous ON of the femoral head at the age of 16 to 18 weeks, while the remaining studies consisted of healthy animals. Characteristics of the included studies are summarised in Tables II to VII. The median of the reported quality score was 3 (2 to 5).

Primary analysis. The overall estimate of the effect of statins was 2.06 (95% CI 1.71 to 2.50, p < 0.001), an approximately two-fold improvement in outcome (Fig. 2). Compared with the control group, the RR was 1.91 (95% CI 1.50 to 2.42) in studies using statins alone while the RR was 2.10 (95% CI 1.68 to 2.62) in studies using statins with other interventions such as bisphosphonate or anticoagulant. Then we further compared the effect of statin exposure alone to that of statins combined with other interventions using data from experiments containing both groups. Results revealed that combined usage significantly decreased the risk of ON (RR = 1.23, 95% CI, 1.02 to 1.47).

Experiments with a relatively low score (< 3 points) gave more conservative estimates of effect size than those with a high score (≥ 3 points) (Fig. 3).

Significant protection by statins against ON was seen for all included species (Fig. 4) but not for gender (RR = 2.24, 1.62 to 3.09 *versus* RR = 2.25, 1.58 to 3.20, p = 0.98).

Effect sizes were similar between studies where statin exposure was started at two weeks before methylprednisolone injection and simultaneously with methylprednisolone administration (Fig. 5). As to outcome measurement, experiments using radiographic examination of ON gave more conservative estimates of effect size (RR = 1.40, 0.97 to 2.02, Fig. 6) than those using histological examination (RR = 2.20, 1.77 to 2.75, Fig.6).

A funnel plot (Fig. 7) analysis showed no evident publication bias towards positive studies, further confirmed by Egger's regression asymmetry test (p > 0.05).

Discussion

This is the first meta-analysis to review the efficacy of statins in preventing GC induced ON in animal models. Our study indicated that animals with statin usage, either alone or combined with other treatment, were at decreased risk of developing GC-related ON. Moreover, subgroup analysis revealed that compared with statin alone, statin usage combined with other treatments significantly decreased the risk of ON (RR = 1.23, 95% CI 1.02 to 1.47). However, we could find no significant risk difference for different gender or for different treatment points (two weeks before or simultaneously with GC injection).

The overall risk estimate of statins in the present study supports the findings of previous work conducted by Pritchett[11] who found that only three patients (3/284, 1%) treated with statins developed ON, much less than the 3% to 20% incidence usually reported for patients receiving high-dose steroids, However, our findings do not correlate with those of the cohort study by Ajmal et al,[10] in which 2881 renal transplantation patients were evaluated and 4.4% (15/338) of patients on statins developed ON compared with 7% (180/2543) of patients who were not on statins. The authors concluded that statin usage does not appear to lower the risk of ON. There are several possible explanations for the discrepancy in the results. Firstly, the dosage of GC administrated to patients following renal transplantation was uncertain at baseline. Higher doses, even of short duration, present greater risks.[2] Secondly, the indication for statin treatment was confined to patients with hypercholesterolaemia. And thirdly, the measure of ON was not reported and it is also unknown whether the measurement was taken identically for each patient.

In a randomised double-blinded placebo-controlled study, Lydonet, Schweitzer and Belmont[30] also found that the incidence of newly developed ON in patients with 40 mg daily of atorvastatin was not statistically different to that found in those administered hydroxychloroquine or bisphosphonates. However, it must be noted that the patients in the control group were administrated bisphosphonates, which may reduce the risk of developing ON to some degree.[4,26,28]

Our results also revealed that statin usage in combination with bisphosphonate or anticoagulant significantly decreased the risk of ON, compared with statins alone, suggesting a protective role for bisphosphonates or anticoagulants against developing GC-induced ON. These findings have been supported elsewhere in the literature.[14,20,28,29,31]

In this current study, we could find no significant difference in risk between animal gender. This differs from the published studies conducted by Ajmal et al,[10] who found that male gender is associated with an increased risk (34%) of ON compared with female patients. The reason for this finding is not clear but it may be related to different mechanisms of fat metabolisms between genders.[32,33]

With regard to animal species, risk estimate was lower in rabbits than in rats. To our knowledge, this has not been reported previously. A possible explanation for this might be that animal models used in Nozaki et al's study[13]

consist of rats with a 50% incidence of spontaneous ON of the femoral head, rather than rats in healthy condition.

Both of the intervention procotols (statin exposure started at two weeks before *versus* simultaneously with methylprednisolone injection) demonstrated protective action against development of ON, but there was no significant difference in effect size between the two groups. To the best of our knowledge, this comparison has not been reported previously. A possible explanation for this finding might be that different statin dosage and species were used across studies. It also may be due to a possible threshold effect for development of ON.[14,29]

As for assessment of ON, it was more conservatively estimated with the use of radiographic examination than with the use of histological examination. Our findings are consistent with those of Kang et al[34] who found no significant changes of ON examined using radiographs during two two to 12-week periods. Accordingly, the difference in risk estimate between various measurements could be explained by the lower detection rate of radiographic examination. Given the small sample size of animals examined using radiographs, however, the results should be considered with caution.

Several limitations must be considered in interpreting our findings. First, the time point at which the outcome was measured varied from study to study. However, ON was evaluated in all included studies at a time point more than two weeks after steroid injection, since two weeks is a time point that has been reported to be crucial in the development of ON.[7,16] Secondly, the sites measured for diagnosis of ON were not controlled for all included studies. The more sites per animal evaluated, the greater risk for the detection of ON. Thirdly, effect size might be weakened in animals with comorbidities Finally, the preventive effects of different statin dosage, and various types of statins on the development of ON, were not analysed.

In conclusion, the present study revealed that statin usage could reduce the risk of GC-related ON in animal models, suggesting statins are efficacious in preventing GC-induced ON to some degree in animals. However, while the overall results of this meta-analysis suggest that statins have substantial effect size, concerns should be raised that the true efficacy of statins might be substantially lower than reported here because of the possible influence of publication or other bias. Furthermore, given the limited application of experimental studies to humans, the heterogeneity among studies and lack of high quality evidence, the results should be extrapolated to the clinical setting with great caution.

Supplementary material

é   A table showing the search strategy is available alongside this article at www.bjr.boneandjoint.org.uk

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